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(21) International Application Number: PCT/IB95/00156 (22) International Filing Date: 23 February 1995 (23.02.95)		(72) Inventors: STINCHCOMB, Dan, T.; 7203 Old Post Road, Boulder, CO 80301 (US). CHOWRIRA, Bharat; 3250 O'Neal Circle, B-25, Boulder, CO 80301 (US). DIRENZO, Anthony; 1197 Ravenwood Road, Boulder, CO 80303 (US). DRAPER, Kenneth, G.; 4619 Cloud Ct., Boulder, CO 80301 (US). DUDYCZ, Lech, W.; 24 A Gates Road, Worcester, MA 01603 (US). GRIMM, Susan; 6968 1/2 S. Boulder Road, Boulder, CO 80303 (US). KARPEISKY, Alexander; 5121 Williams Fork Trail #209, Boulder, CO 80301 (US). KISICH, Kevin; 2451 Jonquil Circle, Lafayette, CO 80026 (US). MATULIC-ADAMIC, Jasenka; 760 South 42nd Street, Boulder, CO 80303 (US). McSWIGGEN, James, A.; 4866 Franklin Drive, Boulder, CO 80301 (US). MODAK, Amit; 3855 Hauptman Court, Boulder, CO 80301 (US). PAVCO, Pamela; 705 Barbary Circle, Lafayette, CO 80026 (US). BEGELMAN, Leonid; 5530 Cok Drive, Longmont, CO 80503 (US). SULLIVAN, Sean, M.; 850 Marina Village Parkway, Alameda, CA 94501 (US). SWEDLER, David; 956 St. Andrews Lane, Louisville, CO 80027 (US). THOMPSON, James, D.; 2925 Glenwood Drive #301, Boulder, CO 80301 (US). TRACZ, Danuta; 6200 Habitat #3029, Boulder, CO 80301 (US). USMAN, Nassim; 2954 Kalmia #37, Boulder, CO 80304 (US). WINCOTT, Francine, E.; 7920 N. 95th Street, Longmont, CO 80501 (US). WOLF, Tod; 18 Fairview Avenue, Watertown, MA 02172 (US).																																																																															
(30) Priority Data: <table border="0"> <tr><td>08/201,109</td><td>23 February 1994 (23.02.94)</td><td>US</td></tr> <tr><td>08/218,934</td><td>29 March 1994 (29.03.94)</td><td>US</td></tr> <tr><td>08/222,795</td><td>4 April 1994 (04.04.94)</td><td>US</td></tr> <tr><td>08/224,483</td><td>7 April 1994 (07.04.94)</td><td>US</td></tr> <tr><td>08/228,041</td><td>15 April 1994 (15.04.94)</td><td>US</td></tr> <tr><td>08/227,958</td><td>15 April 1994 (15.04.94)</td><td>US</td></tr> <tr><td>08/245,736</td><td>18 May 1994 (18.05.94)</td><td>US</td></tr> <tr><td>08/271,280</td><td>6 July 1994 (06.07.94)</td><td>US</td></tr> <tr><td>08/291,932</td><td>15 August 1994 (15.08.94)</td><td>US</td></tr> <tr><td>08/291,433</td><td>16 August 1994 (16.08.94)</td><td>US</td></tr> <tr><td>08/292,620</td><td>17 August 1994 (17.08.94)</td><td>US</td></tr> <tr><td>08/293,520</td><td>19 August 1994 (19.08.94)</td><td>US</td></tr> <tr><td>08/300,000</td><td>2 September 1994 (02.09.94)</td><td>US</td></tr> <tr><td>08/303,039</td><td>8 September 1994 (08.09.94)</td><td>US</td></tr> <tr><td>08/311,486</td><td>23 September 1994 (23.09.94)</td><td>US</td></tr> <tr><td>08/311,749</td><td>23 September 1994 (23.09.94)</td><td>US</td></tr> <tr><td>08/314,397</td><td>28 September 1994 (28.09.94)</td><td>US</td></tr> <tr><td>08/316,771</td><td>3 October 1994 (03.10.94)</td><td>US</td></tr> <tr><td>08/319,492</td><td>7 October 1994 (07.10.94)</td><td>US</td></tr> <tr><td>08/321,993</td><td>11 October 1994 (11.10.94)</td><td>US</td></tr> <tr><td>08/334,847</td><td>4 November 1994 (04.11.94)</td><td>US</td></tr> <tr><td>08/337,608</td><td>10 November 1994 (10.11.94)</td><td>US</td></tr> <tr><td>08/345,516</td><td>28 November 1994 (28.11.94)</td><td>US</td></tr> <tr><td>08/357,577</td><td>16 December 1994 (16.12.94)</td><td>US</td></tr> <tr><td>08/363,233</td><td>23 December 1994 (23.12.94)</td><td>US</td></tr> <tr><td>08/380,734</td><td>30 January 1995 (30.01.95)</td><td>US</td></tr> </table>		08/201,109	23 February 1994 (23.02.94)	US	08/218,934	29 March 1994 (29.03.94)	US	08/222,795	4 April 1994 (04.04.94)	US	08/224,483	7 April 1994 (07.04.94)	US	08/228,041	15 April 1994 (15.04.94)	US	08/227,958	15 April 1994 (15.04.94)	US	08/245,736	18 May 1994 (18.05.94)	US	08/271,280	6 July 1994 (06.07.94)	US	08/291,932	15 August 1994 (15.08.94)	US	08/291,433	16 August 1994 (16.08.94)	US	08/292,620	17 August 1994 (17.08.94)	US	08/293,520	19 August 1994 (19.08.94)	US	08/300,000	2 September 1994 (02.09.94)	US	08/303,039	8 September 1994 (08.09.94)	US	08/311,486	23 September 1994 (23.09.94)	US	08/311,749	23 September 1994 (23.09.94)	US	08/314,397	28 September 1994 (28.09.94)	US	08/316,771	3 October 1994 (03.10.94)	US	08/319,492	7 October 1994 (07.10.94)	US	08/321,993	11 October 1994 (11.10.94)	US	08/334,847	4 November 1994 (04.11.94)	US	08/337,608	10 November 1994 (10.11.94)	US	08/345,516	28 November 1994 (28.11.94)	US	08/357,577	16 December 1994 (16.12.94)	US	08/363,233	23 December 1994 (23.12.94)	US	08/380,734	30 January 1995 (30.01.95)	US	(71) Applicant: RIBOZYME PHARMACEUTICALS, INC. [US/US]; 2950 Wilderness Place, Boulder, CO 80301 (US).	
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NUC 37604

(57) Abstract

Enzymatic RNA molecules which cleave ICAM-1 mRNA, IL-5 mRNA, *ref A* mRNA, TNF- α mRNA, RSV mRNA or RSV genomic RNA, or CML associated mRNA, and use of these molecules for the treatment of pathological conditions related to those mRNA-levels; ribonucleosides or nucleotides modified in 2', 3' or 5', methods for their synthesis, purification and deprotection; vectors containing multiple enzymatic nucleic acids, optionally in chimeric form with tRNAs; method for introducing enzymatic nucleic acids into cells by forming a complex with a second nucleic acid, where the complex is capable of taking an R-loop base-paired structure; method for altering a mutant nucleic acid *in vivo* by hybridization with an oligonucleotide capable of activating dsRNA deaminase, comprising an enzymatic activity or a chemical mutagen. Further are disclosed trans-cleaving or -ligating hairpin ribozymes lacking a substrate RNA moiety, as well as hammerhead ribozymes having an interconnecting loop between base pairs in stem II.

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METHOD AND REAGENT FOR INHIBITING THE EXPRESSION
OF DISEASE RELATED GENESBackground of the Invention

This invention relates to reagents useful as inhibitors of gene expression relating to diseases such as inflammatory or autoimmune disorders, chronic myelogenous leukemia, or respiratory tract illness.

5

Summary of the Invention

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting the expression of disease related genes, e.g., ICAM-1, IL-5, relA, TNF- α , p210 bcr-abl, and respiratory syncytial virus genes. Such ribozymes can be used in a method for
10 treatment of diseases caused by the expression of these genes in man and other animals, including other primates.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. Such enzymatic RNA molecules can be
15 targeted to virtually any RNA transcript, and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Natl. Acad. Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acids Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known
20 presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in trans (and thus can cleave other RNA molecules) under physiological conditions. Table 1 summarizes some of the characteristics of these ribozymes.

Ribozymes act by first binding to a target RNA. Such binding occurs
25 through the target RNA binding portion of a ribozyme which is held in close proximity to an enzymatic portion of the RNA which acts to cleave the target RNA. Thus, the ribozyme first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a
30 target RNA will destroy its ability to direct synthesis of an encoded protein. After a ribozyme has bound and cleaved its RNA target it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule simply binds to a nucleic acid target to block its translation) since the effective concentration of ribozyme necessary to effect a therapeutic treatment is lower than that of an antisense oligonucleotide. The advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding, but also on the mechanism by which the molecule inhibits the expression of the RNA to which it binds. That is, the inhibition is caused by cleavage of the RNA target and so specificity is defined as the ration of the rate of cleavage of the targeted RNA over the rate of cleavage of non-targeted RNA. This cleavage mechanism is dependent upon factors additional to those involved in base pairing. Thus, it is thought that the specificity of action of a ribozyme is greater than that of antisense oligonucleotide binding the same RNA site. With their catalytic activity and increased site specificity, ribozymes represent more potent and safe therapeutic molecules than antisense oligonucleotides.

Thus, in a first aspect, this invention relates to ribozymes, or enzymatic RNA molecules, directed to cleave RNA species encoding ICAM-1, IL-5, relA, TNF- α , p210^{bcr-abl}, or RSV proteins. In particular, applicant describes the selection and function of ribozymes capable of cleaving these RNAs and their use to reduce levels of ICAM-1, IL-5, relA, TNF- α , p210 bor-abl or RSV proteins in various tissues to treat the diseases discussed herein. Such ribozymes are also useful for diagnostic uses.

Applicant indicates that these ribozymes are able to inhibit expression of ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV genes and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave target ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV encoding mRNAs may be readily designed and are within the invention.

These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the

cleavage of RNA. Upon binding, the ribozymes cleave the target encoding mRNAs, preventing translation and protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

By "gene" is meant to refer to either the protein coding regions of the cognate mRNA, or any regulatory regions in the RNA which regulate synthesis of the protein or stability of the mRNA; the term also refers to those regions of an mRNA which encode the ORF of a cognate polypeptide product, and the proviral genome.

By "enzymatic RNA molecule" it is meant an RNA molecule which has complementarity in a substrate binding region to a specified gene target, and also has an enzymatic activity which is active to specifically cleave RNA in that target. That is, the enzymatic RNA molecule is able to intermolecularly cleave RNA and thereby inactivate a target RNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic RNA molecule to the target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. By "equivalent" RNA to a virus is meant to include those naturally occurring viral encoded RNA molecules associated with viral caused diseases in various animals, including humans, cats, simians, and other primates. These viral or viral-encoded RNAs have similar structures and equivalent genes to each other.

By "complementarity" it is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example, Hoogsteen type) of base-paired interactions.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, *Aids Research and Human Retroviruses*, 8,183, of hairpin motifs by Hampel and Tritz, 1989 *Biochemistry*, 28, 4929, EP 0360257 and Hampel *et al.*, 1990, *Nucleic Acids Res.* 18,299 and an example of the hepatitis delta virus motif is described by Perotta and Been, 1992 *Biochemistry*, 31 16 of the RNaseP motif by Guerrier-Takada *et al.*, 1983 *Cell*, 35 849,

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expressed in eukaryotic cells from the appropriate DNA or RNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO93/23569, and Sullivan et al., PCT WO94/02595, both hereby incorporated in their totality by reference herein; Ohkawa, J., et al., 1992, Nucleic Acids Symp. Ser. 27, 15-6; Taira, K. et al., Nucleic Acids Res. 19, 5125-30; Ventura, M., et al., 1993, Nucleic Acids Res. 21, 3249-55, Chowrira et al., 1994 J. Biol. Chem. 269, 25856).

By "inhibit" is meant that the activity or level of ICAM-1, Rel A, IL-5, TNF- α , p210^{bcr-abl} or RSV encoding mRNA is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV protein or activity in a cell or tissue. By "related" is meant that the inhibition of ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV mRNA translation, and thus reduction in the level of, ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV proteins will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues through the use of a catheter, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 2,3,6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36 and 37.

Examples of such ribozymes are shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38. Examples of such ribozymes consist essentially of sequences defined in these Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in the above identified Tables can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequence listed in the above identified Tables may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit ICAM-1, IL-5, Rel A, TNF- α , p210^{bcr-abl} or RSV gene expression are expressed from transcription units inserted into DNA, RNA, or viral vectors. Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA or RNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 *Proc. Natl. Acad. Sci. USA*, 87, 6743-7; Gao and Huang 1993 *Nucleic Acids Res.*, 21, 2867-72; Lieber et al., 1993 *Methods Enzymol.*, 217, 47-66; Zhou et al., 1990 *Mol. Cell. Biol.*, 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 *Antisense Res. Dev.*, 2, 3-15; Ojwang et al., 1992 *Proc. Natl. Acad. Sci. USA*, 90, 6340-4; L'Huillier et al., 1992 *EMBO J.* 11, 4411-8; Lisiewicz et al., 1993 *Proc. Natl. Acad. Sci. U.S.A.*, 90 8000-4). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors

(such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from
 5 the claims.

Description Of The Preferred Embodiments

The drawings will first briefly be described.

Drawings:

Figure 1 is a diagrammatic representation of the hammerhead
 10 ribozyme domain known in the art. Stem II can be ≥ 2 base-pair long.

Figure 2(a) is a diagrammatic representation of the hammerhead
 ribozyme domain known in the art; Figure 2(b) is a diagrammatic
 representation of the hammerhead ribozyme as divided by Uhlenbeck
 (1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure
 15 2(c) is a similar diagram showing the hammerhead divided by Haseloff and
 Gerlach (1988, *Nature*, 334, 585-591) into two portions; and Figure 2(d) is
 a similar diagram showing the hammerhead divided by Jeffries and
 Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a
 20 hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, *n*
 is 1,2,3 or 4) and helix 5 can be optionally provided of length 2 or more
 bases (preferably 3-20 bases, *i.e.*, *m* is from 1-20 or more). Helix 2 and
 helix 5 may be covalently linked by one or more bases (*i.e.*, *r* is ≥ 1 base).
 Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4-20
 25 base pairs) to stabilize the ribozyme structure, and preferably is a protein
 binding site. In each instance, each N and N' independently is any normal
 or modified base and each dash represents a potential base-pairing
 interaction. These nucleotides may be modified at the sugar, base or
 phosphate. Complete base-pairing is not required in the helices, but is
 30 preferred. Helix 1 and 4 can be of any size (*i.e.*, *o* and *p* is each
 independently from 0 to any number, *e.g.* 20) as long as some base-pairing
 is maintained. Essential bases are shown as specific bases in the
 structure, but those in the art will recognize that one or more may be

modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without
5 modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H refers to bases A, U, or C. Y refers to pyrimidine bases. "____" refers to a covalent bond.

Figure 4 is a representation of the general structure of the hepatitis
10 delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a diagrammatic representation of the genetic map of RSV strain A2.

15 Figure 7 is a diagrammatic representation of the solid-phase synthesis of RNA.

Figure 8 is a diagrammatic representation of exocyclic amino protecting groups for nucleic acid synthesis.

Figure 9 is a diagrammatic representation of the deprotection of RNA.

20 Figure 10 is a graphical representation of the cleavage of an RNA substrate by ribozymes synthesized, deprotected and purified using the improved methods described herein.

Figure 11 is a schematic representation of a two pot deprotection protocol. Base deprotection is carried out with aqueous methyl amine at 65
25 °C for 10 min. The sample is dried in a speed-vac for 2-24 hours depending on the scale of RNA synthesis. Silyl protecting group at the 2'-hydroxyl position is removed by treating the sample with 1.4 M anhydrous HF at 65°C for 1.5 hours.

Figure 12 is a schematic representation of a one pot deprotection of
30 RNA synthesized using RNA phosphoramidite chemistry. Anhydrous methyl amine is used to deprotect bases at 65°C for 15 min. The sample is allowed to cool for 10 min before adding TEA•3HF reagent, to the same

pot, to remove protecting groups at the 2'-hydroxyl position. The deprotection is carried out for 1.5 hours.

5 Figs. 13a - b is a HPLC profile of a 36 nt long ribozyme, targeted to site B. The RNA is deprotected using either the two pot or the one pot deprotection protocol. The peaks corresponding to full-length RNA is indicated. The sequence for site B is CCUGGGCCAGGGAUUA AUGGAGAUGCCCACU.

Figure 14 is a graph comparing RNA cleavage activity of ribozymes deprotected by two pot vs one pot deprotection protocols.

10 Figure 15 is a schematic representation of an improved method of synthesizing RNA containing phosphorothioate linkages.

Figure 16 shows RNA cleavage reaction catalyzed by ribozymes containing phosphorothioate linkages. Hammerhead ribozyme targeted to site C is synthesized such that 4 nts at the 5' end contain phosphorothioate linkages. P=O refers to ribozyme without phosphorothioate linkages. P=S refers to ribozyme with phosphorothioate linkages. The sequence for site C is UCAUUUUGGCCAUCUC UUCUUCAGGCGUGG.

Figure 17 is a schematic representation of synthesis of 2'-N-phtalimido-nucleoside phosphoramidite.

20 Figure 18 is a diagrammatic representation of a prior art method for the solid-phase synthesis of RNA using silyl ethers, and the method of this invention using SEM as a 2'-protecting group.

Figure 19 is a diagrammatic representation of the synthesis of 2'-SEM-protected nucleosides and phosphoramidites useful for the synthesis of RNA. B is any nucleotide base as exemplified in the Figure, P is purine and I is inosine. Standard abbreviations are used throughout this application, well known to those in the art.

Figure 20 is a diagrammatic representation of a prior art method for deprotection of RNA using TBDMS protection of the 2'-hydroxyl group.

30 Figure 21 is a diagrammatic representation of the deprotection of RNA having SEM protection of the 2'-hydroxyl group.

Figure 22 is a representation of an HPLC chromatogram of a fully deprotected 10-mer of uridylic acid.

Figs. 23 - 25 are diagrammatic representations of hammerhead, hairpin or hepatitis delta virus ribozyme containing self-processing RNA transcript. Solid arrows indicate self-processing sites. Boxes indicate the sites of nucleotide substitution. Solid lines are drawn to show the binding sites of primers used in a primer-extension assay. Lower case letters indicate vector sequence present in the RNA when transcribed from a *HindIII*-linearized plasmid. (23) HH Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hammerhead ribozyme. The structure of the hammerhead ribozyme is based on phylogenetic and mutational analysis (reviewed by Symons, 1992 supra). The trans ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (UC at positions 50 and 51) at its 3' end. The 3' processing ribozyme is comprised of nucleotides 44 through 96. Roman numerals I, II and III, indicate the three helices that contribute to the structure of the 3' cis-acting hammerhead ribozyme (Hertel et al., 1992 Nucleic Acids Res. 20, 3252). Substitution of G70 and A71 to U and G respectively, inactivates the hammerhead ribozyme (Ruffner et al., 1990 Biochemistry 29, 10695) and generates the HH(mutant) construct. (24) HP Cassette, transcript containing the hammerhead trans-acting ribozyme linked to a 3' cis-acting hairpin ribozyme. The structure of the hairpin ribozyme is based on phylogenetic and mutational analysis (Berzal-Herranz et al., 1993 EMBO J 12, 2567). The trans-ribozyme domain extends from nucleotide 1 through 49. After 3'-end processing, the trans-ribozyme contains 5 non-ribozyme nucleotides (UGGCA at positions 50 to 54) at its 3' end. The 3' cis-acting ribozyme is comprised of nucleotides 50 through 115. The transcript named HP(GU) was constructed with a potential wobble base pair between G52 and U77; HP(GC) has a Watson-Crick base pair between G52 and C77. A shortened helix 1 (5 base pairs) and a stable tetraloop (GAAA) at the end of helix 1 was used to connect the substrate with the catalytic domain of the hairpin ribozyme (Feldstein & Bruening, 1993 Nucleic Acids Res. 21, 1991; Altschuler et al., 1992 supra). (25) HDV Cassette, transcript containing the trans-acting hammerhead ribozyme linked to a 3' cis-acting hepatitis delta virus (HDV) ribozyme. The secondary structure of the HDV ribozyme is as proposed by Been and

coworkers (Been et al., 1992 Biochemistry 31, 11843). The trans-ribozyme domain extends from nucleotides 1 through 48. After 3'-end processing, the trans-ribozyme contains 2 non-ribozyme nucleotides (AA at positions 49 to 50) at its 3' end. The 3' cis-acting HDV ribozyme is comprised of

5 nucleotides 50 through 114. Roman numerals I, II, III & IV, indicate the location of four helices within the 3' cis-acting HDV ribozyme (Perrota & Been, 1991 Nature 350, 434). The Δ HDV transcript contains a 31 nucleotide deletion in the HDV portion of the transcript (nucleotides 84 through 115 deleted).

- 10 Fig. 26 is a schematic representation of a plasmid containing the insert encoding self-processing cassette. The figure is not drawn to scale.

Fig. 27 demonstrates the effect of 3' flanking sequences on RNA self-processing *in vitro*. H, Plasmid templates linearized with *Hind*III restriction enzyme. Transcripts from H templates contain four non-ribozyme

15 nucleotides at the 3' end. N, Plasmid templates linearized with *Nde*I restriction enzyme. Transcripts from N templates contain 220 non-ribozyme nucleotides at the 3' end. R, Plasmid templates linearized with *Rca*I restriction enzyme. Transcripts from R templates contain 450 non-ribozyme nucleotides at the 3' end.

- 20 Fig. 28 shows the effect of 3' flanking sequences on the trans-cleavage reaction catalyzed by a hammerhead ribozyme. A 622 nt internally-labeled RNA (<10 nM) was incubated with ribozyme (1000 nM) under single turn-over conditions (Herschlag and Cech, 1990 Biochemistry 29, 10159). HH+2, HH+37, and HH+52 are trans-acting ribozymes
- 25 produced by transcription from the HH, Δ HDV, and HH(mutant) constructs, respectively, and that contain 2, 37 and 52 extra nucleotides on the 3' end. The plot of the fraction of uncleaved substrate versus time was fit to a double exponential curve using the KaleidaGraph graphing program (Synergy Software, Reading, PA). A double exponential curve fit was used
- 30 because the data points did not fall on a single exponential curve, presumably due to varying conformers of ribozyme and/or substrate RNA.

Fig. 29 shows RNA self-processing in OST7-1 cells. *In vitro* lanes contain full-length, unprocessed transcripts that were added to cellular lysates prior to RNA extraction. These RNAs were either pre-incubated

35 with MgCl₂ (+) or with DEPC-treated water (-) prior to being hybridized

with 5' end-labeled primers. Cellular lanes contain total cellular RNA from cells transfected with one of the four self-processing constructs. Cellular RNA are probed for ribozyme expression using a sequence specific primer-extension assay. Solid arrows indicate the location of primer extension bands corresponding to Full-Length RNA and 3' Cleavage Products.

Figs. 30,31 are diagrammatic representations of self-processing cassettes that will release trans-acting ribozymes with defined, stable stem-loop structures at the 5' and the 3' end following self-processing. 30, shows various permutations of a hammerhead self-processing cassette. 31, shows various permutations of a hairpin self-processing cassette.

Figs. 32a-b Schematic representation of RNA polymerase III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE, refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-acting sequence element that has not been fully characterized. EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

Figs. 33a-e Sequence of the primary tRNA^{met} and Δ3-5 transcripts. The A and B box are internal promoter regions necessary for pol III transcription. Arrows indicate the sites of endogenous tRNA processing. The Δ3-5 transcript is a truncated version of tRNA wherein the sequence 3' of B box has been deleted (Adeniyi-Jones et al., 1984 *supra*). This modification renders the Δ 3-5 RNA resistant to endogenous tRNA processing.

Figure 34. Schematic representation of RNA structural motifs inserted into the Δ3-5 RNA. Δ3-5/HHI- a hammerhead (HHI) ribozyme was cloned at the 3' region of Δ3-5 RNA; S3- a stable stem-loop structure was incorporated at the 3' end of the Δ3-5/HHI chimera; S5- stable stem-loop structures were incorporated at the 5' and the 3' ends of Δ3-5/HHI ribozyme chimera; S35- sequence at the 3' end of the Δ3-5/HHI ribozyme chimera was altered to enable duplex formation between the 5' end and a complementary 3' region of the same RNA; S35Plus- in addition to structural alterations of S35, sequences were altered to facilitate additional

duplex formation within the non-ribozyme sequence of the $\Delta 3$ -5/HHI chimera.

Figures 35 and 36. Northern analysis to quantitate ribozyme expression in T cell lines transduced with $\Delta 3$ -5 vectors. 35) $\Delta 3$ -5/HHI and its variants were cloned individually into the DC retroviral vector (Sullenger et al., 1990 *supra*). Northern analysis of ribozyme chimeras expressed in MT-2 cells was performed. Total RNA was isolated from cells (Chomczynski & Sacchi, 1987 *Analytical Biochemistry* 162, 156-159), and transduced with various constructs described in Fig. 34. Northern analysis was carried out using standard protocols (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Nomenclature is same as in Figure 34. This assay measures the level of expression from the type 2 pol III promoter. 36) Expression of S35 constructs in MT2 cells. S35 (+ribozyme), S35 construct containing HHI ribozyme. S35 (-ribozyme), S35 construct containing no ribozyme.

Figure 37. Ribozyme activity in total RNA extracted from transduced MT-2 cells. Total RNA was isolated from cells transduced with $\Delta 3$ -5 constructs described in Figs. 35 and 36. In a standard ribozyme cleavage reaction, 5 μ g total RNA and trace amounts of 5' terminus-labeled ribozyme target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl₂. RNAs were renatured by cooling the reaction mixture to 37°C for 10-15 min. Cleavage reaction was initiated by mixing the labeled substrate RNA and total cellular RNA at 37°C. The reaction was allowed to proceed for ~ 18h, following which the samples were resolved on a 20 % urea-polyacrylamide gel. Bands were visualized by autoradiography.

Figures 38 and 39. Ribozyme expression and activity levels in S35-transduced clonal CEM cell lines. 38) Northern analysis of S35-transduced clonal CEM cell lines. Standard curve was generated by spiking known concentrations of in vitro transcribed S5 RNA into total cellular RNA isolated from non-transduced CEM cells. Pool, contains RNA from pooled cells transduced with S35 construct. Pool (-G418 for 3 Mo), contains RNA from pooled cells that were initially selected for resistance to G418 and then grown in the absence of G418 for 3 months. Lanes A through N contain RNA from individual clones that were generated from the pooled cells transduced with S35 construct. tRNA_i^{met}, refers to the

endogenous tRNA. S35, refers to the position of the ribozyme band. M, marker lane. 39) Activity levels in S35-transduced clonal CEM cell lines. RNA isolation and cleavage reactions were as described in Fig.37. Nomenclature is same as in Figs. 35 and 36 except, S, 5' terminus-labeled substrate RNA. P, 8 nt 5' terminus-labeled ribozyme-mediated RNA cleavage product.

Figures 40 and 41 are proposed secondary structures of S35 and S35 containing a desired RNA (HHI), respectively. The position of HHI ribozyme is indicated in figure 41. Intramolecular stem refers to the stem structure formed due to an intramolecular base-paired interaction between the 3' sequence and the complementary 5' terminus. The length of the stem ranges from 15-16 base-pairs. Location of the A and the B boxes are shown.

Figures 42 and 43 are proposed secondary structures of S35 plus and S35 plus containing HHI ribozyme.

Figures 44, 45, 46 and 47 are the nucleotide base sequences of S35, HHIS35, S35 Plus, and HHIS35 Plus respectively.

Figs. 48a-b is a general formula for pol III RNA of this invention.

Figure 49 is a digrammatic representation of 5T construct. In this construct the desired RNA is located 3' of the intramolecular stem.

Figures 50 and 51 contain proposed secondary structures of 5T construct alone and 5T construct containing a desired RNA (HHI ribozyme) respectively.

Figure 52 is a diagrammatic representation of TRZ-tRNA chimeras. The site of desired RNA insertion is indicated.

Figure 53 shows the general structure of HHITRZ-A ribozyme chimera. A hammerhead ribozyme targeted to site I is inserted into the stem II region of TRZ-tRNA chimera.

Figure 54 shows the general structure of HPITRZ-A ribozyme chimera. A hairpin ribozyme targeted to site I is cloned into the indicated region of TRZ-tRNA chimera.

Figure 55 shows a comparison of RNA cleavage activity of HHITRZ-A, HHITRZ-B and a chemically synthesized HHI hammerhead ribozymes.

Figure 56 shows expression of ribozymes in T cell lines that are stably transduced with viral vectors. M, markers; lane 1, non-transduced CEM cells; lanes 2 and 3, MT2 and CEM cells transduced with retroviral vectors;
5 lanes 4 and 5, MT2 and CEM cells transduced with AAV vectors.

Figs. 57a-b Schematic diagram of adeno-associated virus and adenoviruses vectors for ribozyme delivery. Both vectors utilize one or more ribozyme encoding transcription units (RZ) based on RNA polymerase II or
10 RNA polymerase III promoters. A. Diagram of an AAV-based vector containing minimal AAV sequences comprising the inverted terminal repeats (ITR) at each end of the vector genome, an optional selectable marker (Neo) driven by an exogenous promoter (Pro), a ribozyme transcription unit, and sufficient additional sequences (stuffer) to maintain a
15 vector length suitable for efficient packaging. B. Diagram of ribozyme expressing adenovirus vectors containing deletions of one or more wild type adenovirus coding regions (cross-hatched boxes marked as E1, pIX, E3, and E4), and insertion of the ribozyme transcription unit at any or several of those regions of deletions.

20 Fig. 58 is a graph showing the effect of arm length variation on the activity of ligated hammerhead (HH) ribozymes. Nomenclature 5/5, 6/6, 7/7, 8/8 and so on refers to the number of base-pairs being formed between the ribozyme and the target. For example, 5/8 means that the HH ribozyme forms 5 bp on the 5' side and 8 bp on the 3' side of the cleavage site for a
25 total of 13 bp. ΔG refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA (Turner and Sugimoto, 1988 Ann. Rev. Biophys. Chem. 17, 167). RPI A is a HH ribozyme with 6/6 binding arms.

30 Figs. 59 and 60 and 61 show cleavage of long substrate (622 nt) by ligated HH ribozymes.

Fig. 62 is a diagrammatic representation of a hammerhead ribozyme (HH-H) targeted against a site termed H. Variants of HH-H are also shown that contain either a 2 base-paired stem II (HH-H1 and HH-H2) or a 3 base-paired stem II (HH-H3 and HH-H4).

Figs. 63 and 64 show RNA cleavage activity of HH-I and its variants (see Fig.62). 63) cleavage of matched substrate RNA (15 nt). 64) cleavage of long substrate RNA (613 nt).

5 Figs. 65a-b is a schematic representation of a method of this invention to synthesize a full length hairpin ribozyme. No splint strand is required for ligation but rather the two fragments hybridize together at helix 4 prior to ligation. The only prerequisite is that the 3' fragment is phosphorylated at its 5' end and that the 3' end of the 5' fragment have a hydroxyl group. The hairpin ribozyme is targeted against site J. H1 and H2 are intermolecular
10 helices formed between the ribozyme and the substrate. H3 and H4 are intramolecular helices formed within the hairpin ribozyme motif. Arrow indicates the cleavage site.

Fig. 66 shows RNA cleavage activity of ligated hairpin ribozymes targeted against site J.

15 Figs. 67a-b is a diagrammatic representation of a Site K Hairpin Ribozyme (HP-K) showing the proposed secondary structure of the hairpin ribozyme-substrate complex as described in the art (Berzal-Herranz *et al.*, 1993 *EMBO. J.*12, 2567). The ribozyme has been assembled from two fragments (bimolecular ribozyme; Chowrira and Burke, 1992 *Nucleic Acids*
20 *Res.* 20, 2835); #H1 and H2 represent intermolecular helix formation between the ribozyme and the substrate. H3 and H4 represent intramolecular helix formation within the ribozyme (intermolecular helix in the case of bimolecular ribozyme). Left panel (HP-K1) indicates 4 base-paired helix 2 and the right panel (HP-K2) indicates 6 base-paired helix 2.
25 Arrow indicates the site of RNA cleavage. All the ribozymes discussed herein were chemically synthesized by solid phase synthesis using RNA phosphoramidite chemistry, unless otherwise indicated. Those skilled in the art will recognize that these ribozymes could also be made transcriptionally *in vitro* and *in vivo*.

30 Figure 68 is a graph showing RNA cleavage by hairpin ribozymes targeted to site K. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-K2' (6 bp helix 2) cleaves a 422 target RNA to a greater extent than the HP-K1 (4 bp helix 2).

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 422 nt region (containing hairpin site A) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α - 32 P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed by chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 422 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Figs. 69a-b is the Site L Hairpin Ribozyme (HP-L) showing proposed secondary structure of the hairpin ribozyme-substrate complex. The ribozyme was assembled from two fragments as described above. The nomenclature is the same as above.

Figure 70 shows RNA cleavage by hairpin ribozymes targeted to site L. A plot of fraction of the target RNA uncleaved (fraction uncleaved) as a function of time is shown. HP-L2 (6 bp helix 2) cleaves a 2 KB target RNA to a greater extent than the HP-L1 (4 bp helix 2). To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 2 kb region (containing hairpin site L) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. The cleavage reactions were carried out as described above.

Figs. 71a-b shows a Site M Hairpin Ribozyme (HP-M) with the proposed secondary structure of the hairpin ribozyme-substrate complex. The ribozyme was assembled from two fragments as described above.

- Figure 72 is a graph showing RNA cleavage by hairpin ribozymes targeted to site M. The ribozymes were tested at both 20°C and at 26°C. To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.9 KB region (containing hairpin site M) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Cleavage reactions were carried out as described above except that 20°C and at 26°C temperatures were used.

- Figs. 73a-d shows various structural modifications of the present invention. A) Hairpin ribozyme lacking helix 5. Nomenclature is same as described under figure 3. B) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by a nucleotide loop wherein q is ≥ 2 bases. Nomenclature is same as described under figure 3. C) Hairpin ribozyme lacking helix 5. Helix 4 loop is replaced by a linker 10^3L , wherein L is a non-nucleotide linker molecule (Benseler *et al.*, 1993 *J. Am. Chem. Soc.* 115, 8483; Jennings *et al.*, WO 94/13688). Nomenclature is same as described under figure 3. D) Hairpin ribozyme lacking helix 4 and helix 5. Helix 4 is replaced by non-nucleotide linker molecule " L " (Benseler *et al.*, 1993 *supra*; Jennings *et al.*, *supra*). Nomenclature is same as described under figure 3.

- Figs. 74a-b shows Hairpin ribozymes containing nucleotide spacer region " s " at the indicated location, wherein s is ≥ 1 base. Hairpin ribozymes containing spacer region, can be synthesized as one fragment or can be assembled from multiple fragments. Nomenclature is same as described under figure 3.

- Figs. 75a-e shows the structures of the 5'-C-alkyl-modified nucleotides. R_1 is as defined above. R is OH, H, O-protecting group, NH, or any group described by the publications discussed above, and those described below. B is as defined in the Figure or any other equivalent nucleotide base. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 76 is a diagrammatic representation of the synthesis of 5'-C-alkyl-D-allose nucleosides and their phosphoramidites.

Figure 77 is a diagrammatic representation of the synthesis of 5'-C-alkyl-L-talose nucleosides and their phosphoramidites.

- 5 Figure 78 is a diagrammatic representation of hammerhead ribozymes targeted to site O containing 5'-C-methyl-L-talo modifications at various positions.

Figure 79 shows RNA cleavage activity of HH-O ribozymes. Fraction of target RNA uncleaved as a function of time is shown.

- 10 Figure 80 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* 1992, 20, 3252) showing specific substitutions.

- 15 Figs. 81a-j shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 82 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

Figure 83 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene uridine.

- 20 Figure 84 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 85 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

- 25 Figure 86 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidene uridine, 2'-C-methoxycarboxymethylidene uridine and derivatized amidites thereof. X is CH₃ or alkyl as discussed above, or another substituent.

Figure 87 is a diagrammatic representation of a synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonates.

Figure 88 is a diagrammatic representation of the synthesis of nucleoside 5'-deoxy-5'-difluoromethylphosphonate 3'-phosphoramidites, dimers and solid supported dimers.

Figure 89 is a diagrammatic representation of the synthesis of
5 nucleoside 5'-deoxy-5'-difluoromethylene triphosphates.

Figures 90 and 91 are diagrammatic representations of the synthesis of 3'-deoxy-3'-difluoromethylphosphonates and dimers.

Figure 92 is a schematic representation of synthesizing RNA phosphoramidite of a nucleotide containing a 2'-hydroxyl group
10 modification of the present invention.

Figs. 93a-b describes a method for deprotection of oligonucleotides containing a 2'-hydroxyl group modification of the present invention.

Figure 94 is a diagrammatic representation of a hammerhead ribozyme targeted to site N. Positions of 2'-hydroxyl group substitution is
15 indicated.

Figure 95 shows RNA cleavage activity of ribozymes containing a 2'-hydroxyl group modification of the present invention. All RNA, represents hammerhead ribozyme (HHN) with no 2'-hydroxyl group modifications. U7-ala, represents HHN ribozyme containing 2'-NH-alanine modification at the
20 U7 position. U4/U7-ala, represents HHA containing 2'-NH-alanine modifications at U4 and U7 positions. U4 lys, represents HHA containing 2'-NH-lysine modification at U4 position. U7 lys, represents HHA containing 2'-NH-lysine modification at U7 position. U4/U7-lys, represents HHN containing 2'-NH-lysine modification at U4 and U7 positions.

25 Figures 96 and 97 are schematic representations of synthesizing (solid-phase synthesis) 3' ends of RNA with modification of the present invention. B, refers to either a base, modified base or an H.

Figure 98 and 99 are schematic representations of synthesizing (solid-phase synthesis) 5' ends of RNA with modification of the present
30 invention. B, refers to either a base, modified base or an H.

Figures 100 and 101 are general schematic representations of the invention.

Fig. 102a-d is a schematic representation of a method of the invention.

Fig. 103 is a graph of the results of the experiment diagrammed in figure 104.

Figure 104 is a diagrammatic representation of a fusion mRNA used
5 in the experiment diagrammed in Fig. 102.

Figure 105 is a diagrammatic representation of a method for selection of useful ribozymes of this invention.

Figure 106 generally shows R-loop formation, and an R-loop complex. In addition, it indicates the location at which ligands can be
10 provided to target the R-loop complex to cells using at least three different procedures, such as ligand receptor interaction, lipid or calcium phosphate mediated delivery, or electroporation.

Figure 107 shows a method for use of self-processing ribozymes to generate therapeutic ribozymes of unit length. This method is essentially
15 described by Draper et al., PCT WO 93/23509.

Figure 108 shows a method of linking ligands like folate, carbohydrate or peptides to R-loop forming RNA.

Ribozymes of this invention block to some extent ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV genes expression and can be used to treat
20 diseases or diagnose such diseases. Ribozymes will be delivered to cells in culture and to tissues in animal models. Ribozyme cleavage of ICAM-1, IL-5, rel A, TNF- α , p210^{bcr-abl}, or RSV mRNA in these systems may prevent or alleviate disease symptoms or conditions.

I. Target sites

25 Targets for useful ribozymes can be determined as disclosed in Draper et al PCT WO93/23509, Sullivan *et al.*, PCT WO94/02595 as well as by Draper et al., PCT/US94/13129 and hereby incorporated by reference herein in totality. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such
30 methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be

optimized and delivered as described therein. While specific examples to animal and human RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for
5 targeting human RNA sequences are present in the ribozyme. Such targets may also be selected as described below.

It must be established that the sites predicted by the computer-based RNA folding algorithm correspond to potential cleavage sites. Hammerhead or hairpin ribozymes are designed that could bind and are
10 individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci., USA, 86 7706-7710) to assess whether the ribozyme sequences fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm
15 lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

mRNA is screened for accessible cleavage sites by the method described generally in Draper et al., PCT WO93/23569 hereby incorporated by reference herein. Briefly, DNA oligonucleotides
20 representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to generate a substrate for T7 RNA polymerase transcription from cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from DNA templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is
25 added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a phosphor imaging system. From these data, hammerhead or hairpin ribozyme sites are chosen as the
30 most accessible.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used
35 follows the procedure for normal RNA synthesis as described in Usman et al., 1987 *J. Am. Chem. Soc.*, 109, 7845 and in Scaringe et al., 1990

Nucleic Acids Res., 18, 5433 and made use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, phosphoramidites at the 3'-end. The average stepwise coupling yields are >98%. Inactive ribozymes are synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from Hertel et al., 1992 *Nucleic Acids Res.*, 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840). Ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbach, 1989, *Methods Enzymol*, 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17,34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography and are resuspended in water.

Example 1: ICAM-1

Ribozymes that cleave ICAM-1 mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. ICAM-1 function can be blocked therapeutically using monoclonal antibodies. Ribozymes have the advantage of being generally immunologically inert, whereas significant neutralizing anti-IgG responses can be observed with some monoclonal antibody treatments.

The following is a brief description of the physiological role of ICAM-1. The discussion is not meant to be complete and is provided only for understanding of the invention that follows. This summary is not an admission that any of the work described below is prior art to the claimed invention.

Intercellular adhesion molecule-1 (ICAM-1) is a cell surface protein whose expression is induced by inflammatory mediators. ICAM-1 is required for adhesion of leukocytes to endothelial cells and for several immunological functions including antigen presentation, immunoglobulin production and cytotoxic cell activity. Blocking ICAM-1 function prevents immune cell recognition and activity during transplant rejection and in animal models of rheumatoid arthritis, asthma and reperfusion injury.

- Cell-cell adhesion plays a pivotal role in inflammatory and immune responses (Springer et al., 1987 *Ann. Rev. Immunol.* 5, 223-252). Cell adhesion is required for leukocytes to bind to and migrate through vascular endothelial cells. In addition, cell-cell adhesion is required for antigen presentation to T cells, for B cell induction by T cells, as well as for the cytotoxicity activity of T cells, NK cells, monocytes or granulocytes. Intercellular adhesion molecule-1 (ICAM-1) is a 110 kilodalton member of the immunoglobulin superfamily that is involved in all of these cell-cell interactions (Simmons et al., 1988 *Nature (London)* 331, 624-627).
- 10 ICAM-1 is expressed on only a limited number of cells and at low levels in the absence of stimulation (Dustin et al., 1986 *J. Immunol.* 137, 245-254). Upon treatment with a number of inflammatory mediators (lipopolysaccharide, γ -interferon, tumor necrosis factor- α , or interleukin-1), a variety of cell types (endothelial, epithelial, fibroblastic and hematopoietic cells) in a variety of tissues express high levels of ICAM-1 on their surface (Springer et al. *supra*; Dustin et al., *supra*; and Rothlein et al., 1988 *J. Immunol.* 141, 1665-1669). Induction occurs via increased transcription of ICAM-1 mRNA (Simmons et al., *supra*). Elevated expression is detectable after 4 hours and peaks after 16 - 24 hours of induction.
- 20 ICAM-1 induction is critical for a number of inflammatory and immune responses. *In vitro*, antibodies to ICAM-1 block adhesion of leukocytes to cytokine-activated endothelial cells (Boyd, 1988 *Proc. Natl. Acad. Sci. USA* 85, 3095-3099; Dustin and Springer, 1988 *J. Cell Biol.* 107, 321-331). Thus, ICAM-1 expression may be required for the extravasation of immune cells to sites of inflammation. Antibodies to ICAM-1 also block T cell killing, mixed lymphocyte reactions, and T cell-mediated B cell differentiation, suggesting that ICAM-1 is required for these cognate cell interactions (Boyd et al., *supra*). The importance of ICAM-1 in antigen presentation is underscored by the inability of ICAM-1 defective murine B cell mutants to stimulate antigen-dependent T cell proliferation (Dang et al., 1990 *J. Immunol.* 144, 4082-4091). Conversely, murine L cells require transfection with human ICAM-1 in addition to HLA-DR in order to present antigen to human T cells (Altmann et al., 1989 *Nature (London)* 338, 512-514). In summary, evidence *in vitro* indicates that ICAM-1 is required for cell-cell interactions critical to inflammatory responses, cellular immune responses, and humoral antibody responses.
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By engineering ribozyme motifs we have designed several ribozymes directed against ICAM-1 mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance. These ribozymes cleave ICAM-1 target sequences *in vitro*.

- 5 The sequence of human, rat and mouse ICAM-1 mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Tables 2, 3, and 6-9. (All sequences
10 are 5' to 3' in the tables) While rat, mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility.

- The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 4 - 8 and 10. Those in the art will recognize that
15 these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

- 20 The ribozymes will be tested for function *in vivo* by exogenous delivery to human umbilical vein endothelial cells (HUVEC). Ribozymes will be delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors described above. Cytokine-induced ICAM-1 expression will be
25 monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. ICAM-1 mRNA levels will be assessed by Northern, by RNase protection, by primer extension or by quantitative RT-PCR analysis. Ribozymes that block the induction of ICAM-1 protein and mRNA by more than 90% will be identified.

- 30 As disclosed by Sullivan et al., PCT WO94/02595, incorporated by reference herein, ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of ICAM-1 mRNA and protein. The effect of the anti-ICAM-1 ribozymes on graft
35 rejection will then be assessed. Similarly, ribozymes will be introduced

into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-ICAM-1 ribozyme or a gene
5 construct that constitutively expresses the ribozyme may abrogate inflammatory and immune responses in these diseases.

Uses

ICAM-1 plays a central role in immune cell recognition and function. Ribozyme inhibition of ICAM-1 expression can reduce transplant rejection
10 and alleviate symptoms in patients with rheumatoid arthritis, asthma or other acute and chronic inflammatory disorders. We have engineered several ribozymes that cleave ICAM-1 mRNA. Ribozymes that efficiently inhibit ICAM-1 expression in cells can be readily found and their activity measured with regard to their ability to block transplant rejection and
15 arthritis symptoms in animal models. These anti-ICAM-1 ribozymes represent a novel therapeutic for the treatment of immunological or inflammatory disorders.

The therapeutic utility of reduction of activity of ICAM-1 function is evident in the following disease targets. The noted references indicate the
20 role of ICAM-1 and the therapeutic potential of ribozymes described herein. Thus, these targets can be therapeutically treated with agents that reduce ICAM-1 expression or function. These diseases and the studies that support a critical role for ICAM-1 in their pathology are listed below. This list is not meant to be complete and those in the art will recognize further
25 conditions and diseases that can be effectively treated using ribozymes of the present invention.

- Transplant rejection

ICAM-1 is expressed on venules and capillaries of human cardiac biopsies with histological evidence of graft rejection (Briscoe et al., 1991 *Transplantation*
30 51, 537-539).

Antibody to ICAM-1 blocks renal (Cosimi et al., 1990 *J. Immunol.* 144, 4604-4612) and cardiac (Flavin et al., 1991 *Transplant. Proc.* 23, 533-534) graft rejection in primates.

A Phase I clinical trial of a monoclonal anti-ICAM-1 antibody showed significant reduction in rejection and a significant increase in graft function in human kidney transplant patients (Haug, et al., 1993 *Transplantation* 55, 766-72).

- Rheumatoid arthritis

- 5 ICAM-1 overexpression is seen on synovial fibroblasts, endothelial cells, macrophages, and some lymphocytes (Chin et al., 1990 *Arthritis Rheum* 33, 1776-86; Koch et al., 1991 *Lab Invest* 64, 313-20).

Soluble ICAM-1 levels correlate with disease severity (Mason et al., 1993 *Arthritis Rheum* 36, 519-27).

- 10 Anti-ICAM antibody inhibits collagen-induced arthritis in mice (Kakimoto et al., 1992 *Cell Immunol* 142, 326-37).

Anti-ICAM antibody inhibits adjuvant-induced arthritis in rats (Iigo et al., 1991 *J Immunol* 147, 4167-71).

- Myocardial ischemia, stroke, and reperfusion injury

- 15 Anti-ICAM-1 antibody blocks adherence of neutrophils to anoxic endothelial cells (Yoshida et al., 1992 *Am J Physiol* 262, H1891-8).

Anti-ICAM-1 antibody reduces neurological damage in a rabbit model of cerebral stroke (Bowes et al., 1993 *Exp Neurol* 119, 215-9).

- 20 Anti-ICAM-1 antibody protects against reperfusion injury in a cat model of myocardial ischemia (Ma et al., 1992 *Circulation* 86, 937-46).

- Asthma

Antibody to ICAM-1 partially blocks eosinophil adhesion to endothelial cells and is overexpressed on inflamed airway endothelium and epithelium *in vivo* (Wegner et al., 1990 *Science* 247, 456-9).

- 25 In a primate model of asthma, anti-ICAM-1 antibody blocks airway eosinophilia (Wegner et al., *supra*) and prevents the resurgence of airway inflammation and hyper-responsiveness after dexamethasone treatment (Gundel et al., 1992 *Clin Exp Allergy* 22, 569-75).

- Psoriasis

Surface ICAM-1 and a clipped, soluble version of ICAM-1 is expressed in psoriatic lesions and expression correlates with inflammation (Kellner et al., 1991 *Br J Dermatol* 125, 211-6; Griffiths 1989 *J Am Acad Dermatol* 20, 617-29; Schopf et al., 1993 *Br J Dermatol* 128, 34-7).

- 5 Anti-ICAM antibody blocks keratinocyte antigen presentation to T cells (Nickoloff et al., 1993 *J Immunol* 150, 2148-59).

- Kawasaki disease

Surface ICAM-1 expression correlates with the disease and is reduced by effective immunoglobulin treatment (Leung, et al., 1989 *Lancet* 2, 1298-302).

- 10 Soluble ICAM levels are elevated in Kawasaki disease patients; particularly high levels are observed in patients with coronary artery lesions (Furukawa et al., 1992 *Arthritis Rheum* 35, 672-7; Tsuji, 1992 *Aterugi* 41, 1507-14).

- 15 Circulating LFA-1⁺ T cells are depleted (presumably due to ICAM-1 mediated extravasation) in Kawasaki disease patients (Furukawa et al., 1993 *Scand J Immunol* 37, 377-80).

Example 2: IL-5

- 20 Ribozymes that cleave IL-5 mRNA represent a novel therapeutic approach to inflammatory disorders like asthma. The invention features use of ribozymes to treat chronic asthma, e.g. by inhibiting the synthesis of IL-5 in lymphocytes and preventing the recruitment and activation of eosinophils.

- 25 A number of cytokines besides IL-5 may also be involved in the activation of inflammation in asthmatic patients, including platelet activating factor, IL-1, IL-3, IL-4, GM-CSF, TNF- α , gamma interferon, VCAM, ILAM-1, ELAM-1 and NF- κ B. In addition to these molecules, it is appreciated that any cellular receptors which mediate the activities of the cytokines are also good targets for intervention in inflammatory diseases. These targets include, but are not limited to, the IL-1R and TNF- α R on keratinocytes, epithelial and endothelial cells in airways. Recent data suggest that certain
- 30 neuropeptides may play a role in asthmatic symptoms. These peptides include substance P, neurokinin A and calcitonin-gene-related peptides. These target genes may have more general roles in inflammatory diseases, but are currently assumed to have a role only in asthma.

Ribozymes of this invention block to some extent IL-5 expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of asthma (Clutterbuck et al., 1989 supra; Garssen et al., 1991 Am. Rev. Respir. Dis. 144, 931-938; Larsen et al., 1992 J. Clin. Invest. 89, 747-752; Mauser et al., 1993 supra). Ribozyme cleavage of IL-5 mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse IL-5 mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 11, 13, and 14, 15. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 12, lower case letters indicate positions that are not conserved between the Human and the Mouse IL-5 sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 12, 14 - 16. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables 12 and 14 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 15 and 16 (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 12, 14 - 16 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against IL-5 mRNA sequences. These ribozymes are synthesized

with modifications that improve their nuclease resistance. The ability of ribozymes to cleave IL-5 target sequences *in vitro* is evaluated.

- The ribozymes will be tested for function *in vivo* by analyzing IL-5 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA or RNA vectors. IL-5 expression will be monitored by biological assays, ELISA, by indirect immunofluorescence, and/or by FACS analysis. IL-5 mRNA levels will be assessed by Northern analysis, RNase protection or primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of IL-5 activity and/or IL-5 mRNA by more than 90% will be identified.

Uses

- Interleukin 5 (IL-5), a cytokine produced by CD4+ T helper cells and mast cells, was originally termed B cell growth factor II (reviewed by Takatsu et al., 1988 Immunol. Rev. 102, 107). It stimulates proliferation of activated B cells and induces production of IgM and IgA. IL-5 plays a major role in eosinophil function by promoting differentiation (Clutterbuck et al., 1989 Blood 73, 1504-12), vascular adhesion (Walsh et al., 1990 Immunology 71, 258-65) and *in vitro* survival of eosinophils (Lopez et al., 1988 J. Exp. Med. 167, 219-24). This cytokine also enhances histamine release from basophils (Hirai et al., 1990 J. Exp. Med. 172, 1525-8). The following summaries of clinical results support the selection of IL-5 as a primary target for the treatment of asthma:

- Several studies have shown a direct correlation between the number of activated T cells and the number of eosinophils from asthmatic patients vs. normal patients (Oehling et al., 1992 J. Investig. Allergol. Clin. Immunol. 2, 295-9). Patients with either allergic asthma or intrinsic asthma were treated with corticosteroids. The bronchoalveolar lavage was monitored for eosinophils, activated T helper cells and recovery of pulmonary function over a 28 to 30 day period. The number of eosinophils and activated T helper cells decreased progressively with subsequent improvement in pulmonary function compared to intrinsic asthma patients with no corticosteroid treatment.

- Bronchoalveolar lavage cells were screened for production of cytokines using *in situ* hybridization for mRNA. *In situ* hybridization signals

were detected for IL-2, IL-3, IL-4, IL-5 and GM-CSF. Upregulation of mRNA was observed for IL-4, IL-5 and GM-CSF (Robinson et al., 1993 J. Allergy Clin. Immunol. 92, 313-24). Another study showed that upregulation of IL-5 transcripts from allergen challenged vs. saline challenged asthmatic patients (Krishnaswamy et al., 1993 Am. J. Respir. Cell. Mol. Biol. 9, 279-86).

An 18 patient study was performed to determine a mechanism of action for corticosteroid improvement of asthma symptoms. Improvement was monitored by methacholine responsiveness. A correlation was observed between the methacholine responsiveness, a reduction in the number of eosinophils, a reduction in the number of cells expressing IL-4 and IL-5 mRNA and an increase in number of cells expressing interferon-gamma.

Bronchial biopsies from 15 patients were analyzed 24 hours after allergen challenge (Bentley et al., 1993 Am. J. Respir. Cell. Mol. Biol. 8, 35-42). Increased numbers of eosinophils and IL-2 receptor positive cells were found in the biopsies. No differences in the numbers of total leukocytes, T lymphocytes, elastase-positive neutrophils, macrophages or mast cell subtypes were observed. The number of cells expressing IL-5 and GM-CSF mRNA significantly increased.

In another patient study, the eosinophil phenotype was the same for asthmatic patients and normal individuals. However, eosinophils from asthmatic patients had greater leukotriene C4 producing capacity and migration capacity. There were elevated levels of IL-3, IL-5 and GM-CSF in the circulation of asthmatics but not in normal individuals (Bruijnzeel et al., 1992 Schweiz. Med. Wochenschr. 122, 298-301).

Efficacy of antibody to IL-5 was assessed in a guinea pig asthma model. The animals were challenged with ovalbumin and assayed for eosinophilia and the responsiveness to the bronchioconstriction substance P. A 30 mg/kg dose of antibody administered i.p. blocked ovalbumin-induced increased sensitivity to substance P and blocked increases in bronchoalveolar and lung tissue accumulation of eosinophils (Mausser et al., 1993 Am. Rev. Respir. Dis. 148, 1623-7). In a separate study guinea pigs challenged for eight days with ovalbumin were treated with monoclonal antibody to IL-5. Treatment produced a reduction in the

number of eosinophils in bronchoalveolar lavage. No reduction was observed for unchallenged guinea pigs and guinea pigs treated with a control antibody. Antibody treatment completely inhibited the development of hyperreactivity to histamine and arecoline after ovalbumin challenge

5 (van Oosterhout et al., 1993 Am. Rev. Respir. Dis. 147, 548-52)

Results obtained from human clinical analysis and animal studies indicate the role of activated T helper cells, cytokines and eosinophils in asthma. The role of IL-5 in eosinophil development and function makes IL-5 a good candidate for target selection. The antibody studies neutralized

10 IL-5 in the circulation thus preventing eosinophilia. Inhibition of the production of IL-5 will achieve the same goal.

Asthma – a prominent feature of asthma is the infiltration of eosinophils and deposition of toxic eosinophil proteins (e.g. major basic protein, eosinophil-derived neurotoxin) in the lung. A number of T-cell-

15 derived factors like IL-5 are responsible for the activation and maintenance of eosinophils (Kay, 1991 J. Allergy Clin. Immun. 87, 893). Inhibition of IL-5 expression in the lungs can decrease the activation of eosinophils and will help alleviate the symptoms of asthma.

Atopy – is characterized by the development of type I hypersensitive reactions associated with exposure to certain environmental antigens. One

20 of the common clinical manifestations of atopy is eosinophilia (accumulation of abnormally high levels of eosinophils in the blood). Antibodies against IL-5 have been shown to lower the levels of eosinophils in mice (Cook et al., 1993 in Immunopharmacol. Eosinophils ed. Smith and

25 Cook, pp. 193-216, Academic, London, UK)

Parasitic infection-related eosinophilia– infections with parasites like helminths, can lead to severe eosinophilia (Cook et al., 1993 supra). Animal models for eosinophilia suggest that infection of mice, for

30 example, can lead to blood, peritoneal and/or tissue eosinophilia, all of which seem to be lowered to varying degrees by antibodies directed against IL-5.

Pulmonary infiltration eosinophilia– is characterised by accumulation of high levels of eosinophils in pulmonary parenchyma (Gleich, 1990 J. Allergy Clin. Immunol. 85, 422).

L-Tryptophan-associated eosinophilia-myalgia syndrome (EMS)— The EMS disease is closely linked to the consumption of L-tryptophan, an essential aminoacid used to treat conditions like insomnia (for review see Varga et al., 1993 J Invest. Dermatol. 100, 97s). Pathologic and histologic studies have demonstrated high levels of eosinophils and mononuclear inflammatory cells in patients with EMS. It appears that IL-5 and transforming growth factor play a significant role in the development of EMS (Varga et al., 1993 supra) by activating eosinophils and other inflammatory cells.

Thus, ribozymes of the present invention that cleave IL-5 mRNA and thereby IL-5 activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits IL-5 function is described above; available cellular and activity assays are numerous, reproducible, and accurate. Animal models for IL-5 function and for each of the suggested disease targets exist (Cook et al., 1993 supra) and can be used to optimize activity.

Example 3: NF- κ B

Ribozymes that cleave *rel A* mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders. Inflammatory mediators such as lipopolysaccharide (LPS), interleukin-1 (IL-1) or tumor necrosis factor- α (TNF- α) act on cells by inducing transcription of a number of secondary mediators, including other cytokines and adhesion molecules. In many cases, this gene activation is known to be mediated by the transcriptional regulator, NF- κ B. One subunit of NF- κ B, the *rel A* gene product (termed RelA or p65) is implicated specifically in the induction of inflammatory responses. Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target intracellular factors that contribute to disease pathology. Thus, ribozymes that cleave mRNA encoded by *rel A* or TNF- α may represent novel therapeutics for the treatment of inflammatory and autoimmune disorders.

The nuclear DNA-binding activity, NF- κ B, was first identified as a factor that binds and activates the immunoglobulin κ light chain enhancer in B cells. NF- κ B now is known to activate transcription of a variety of other cellular genes (e.g., cytokines, adhesion proteins, oncogenes and viral

proteins) in response to a variety of stimuli (e.g., phorbol esters, mitogens, cytokines and oxidative stress). In addition, molecular and biochemical characterization of NF- κ B has shown that the activity is due to a homodimer or heterodimer of a family of DNA binding subunits. Each subunit bears a stretch of 300 amino acids that is homologous to the oncogene, *v-rel*. The activity first described as NF- κ B is a heterodimer of p49 or p50 with p65. The p49 and p50 subunits of NF- κ B (encoded by the *nf- κ B2* or *nf- κ B1* genes, respectively) are generated from the precursors NF- κ B1 (p105) or NF- κ B2 (p100). The p65 subunit of NF- κ B (now termed Rel A) is encoded by the *rel A* locus.

The roles of each specific transcription-activating complex now are being elucidated in cells (N.D. Perkins, et al., 1992 Proc. Natl Acad. Sci USA 89, 1529-1533). For instance, the heterodimer of NF- κ B1 and Rel A (p50/p65) activates transcription of the promoter for the adhesion molecule, VCAM-1, while NF- κ B2/RelA heterodimers (p49/p65) actually inhibit transcription (H.B. Shu, et al., *Mol. Cell. Biol.* 13, 6283-6289 (1993)). Conversely, heterodimers of NF- κ B2/RelA (p49/p65) act with Tat-I to activate transcription of the HIV genome, while NF- κ B1/RelA (p50/p65) heterodimers have little effect (J. Liu, N.D. Perkins, R.M. Schmid, G.J. Nabel, *J. Virol.* 1992 66, 3883-3887). Similarly, blocking *rel A* gene expression with antisense oligonucleotides specifically blocks embryonic stem cell adhesion; blocking NF- κ B1 gene expression with antisense oligonucleotides had no effect on cellular adhesion (Narayanan et al., 1993 Mol. Cell. Biol. 13, 3802-3810). Thus, the promiscuous role initially assigned to NF- κ B in transcriptional activation (M.J. Lenardo, D. Baltimore, 1989 Cell 58, 227-229) represents the sum of the activities of the *rel* family of DNA-binding proteins. This conclusion is supported by recent transgenic "knock-out" mice of individual members of the *rel* family. Such "knock-outs" show few developmental defects, suggesting that essential transcriptional activation functions can be performed by more than one member of the *rel* family.

A number of specific inhibitors of NF- κ B function in cells exist, including treatment with phosphorothioate antisense oligonucleotide, treatment with double-stranded NF- κ B binding sites, and over expression of the natural inhibitor MAD-3 (an I κ B family member). These agents have

been used to show that NF- κ B is required for induction of a number of molecules involved in inflammation, as described below.

- NF- κ B is required for phorbol ester-mediated induction of IL-6 (I. Kitajima, et al., Science 258, 1792-5 (1992)) and IL-8 (Kunsch and Rosen, 1993 Mol. Cell. Biol. 13, 6137-46).

•NF- κ B is required for induction of the adhesion molecules ICAM-1 (Eck, et al., 1993 Mol. Cell. Biol. 13, 6530-6536), VCAM-1 (Shu et al., *supra*), and E-selectin (Read, et al., 1994 J. Exp. Med. 179, 503-512) on endothelial cells.

- 10 •NF- κ B is involved in the induction of the integrin subunit, CD18, and other adhesive properties of leukocytes (Eck et al., 1993 *supra*).

The above studies suggest that NF- κ B is integrally involved in the induction of cytokines and adhesion molecules by inflammatory mediators. Two recent papers point to another connection between NF- κ B and inflammation: glucocorticoids may exert their anti-inflammatory effects by inhibiting NF- κ B. The glucocorticoid receptor and p65 both act at NF- κ B binding sites in the ICAM-1 promoter (van de Stolpe, et al., 1994 J. Biol. Chem. 269, 6185-6192). Glucocorticoid receptor inhibits NF- κ B-mediated induction of IL-6 (Ray and Prefontaine, 1994 Proc. Natl Acad. Sci USA 91, 752-756). Conversely, overexpression of p65 inhibits glucocorticoid induction of the mouse mammary tumor virus promoter. Finally, protein cross-linking and co-immunoprecipitation experiments demonstrated direct physical interaction between p65 and the glucocorticoid receptor (*Id.*).

- 25 Ribozymes of this invention block to some extent NF- κ B expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of restenosis, transplant rejection and rheumatoid arthritis. Ribozyme cleavage of *relA* mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

- 30 The sequence of human and mouse *relA* mRNA can be screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 17, 18 and 21-22. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and

ribozymes thereafter designed, the human targetted sequences are of most utility.

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 19 - 22. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity and may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

- 10 By engineering ribozyme motifs we have designed several ribozymes directed against *rel A* mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave *rel A* target sequences *in vitro* is evaluated.

- 15 The ribozymes will be tested for function *in vivo* by analyzing cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA and RNA vectors. Cytokine-induced VCAM-1, ICAM-1, IL-6 and IL-8 expression will be monitored by ELISA, by indirect immunofluoresence, and/or by FACS analysis. *Rel A* mRNA levels will be assessed by Northern analysis, RNAse protection or primer extension analysis or quantitative RT-PCR. Activity of NF- κ B will be monitored by gel-retardation assays. Ribozymes that block the induction of NF- κ B activity and/or *rel A* mRNA by more than 50% will be identified.

- 25 RNA ribozymes and/or genes encoding them will be locally delivered to transplant tissue *ex vivo* in animal models. Expression of the ribozyme will be monitored by its ability to block *ex vivo* induction of VCAM-1, ICAM-1, IL-6 and IL-8 mRNA and protein. The effect of the anti-*rel A* ribozymes on graft rejection will then be assessed. Similarly, ribozymes will be introduced into joints of mice with collagen-induced arthritis or rabbits with *Streptococcal* cell wall-induced arthritis. Liposome delivery, cationic lipid delivery, or adeno-associated virus vector delivery can be used. One dose (or a few infrequent doses) of a stable anti-*rel A* ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate inflammatory and immune responses in these diseases.
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Uses

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves *rel A* mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

•Rheumatoid arthritis (RA).

Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, J. Clin. Invest. 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Restenosis.

Expression of NF- κ B in the vessel wall of pigs causes a narrowing of the luminal space due to excessive deposition of extracellular matrix components. This phenotype is similar to matrix deposition that occurs subsequent to coronary angioplasty. In addition, NF- κ B is required for the expression of the oncogene *c-myc* (F.A. La Rosa, J.W. Pierce, G.E. Sonenshein, Mol. Cell. Biol. 14, 1039-44 (1994)). Thus NF- κ B induces smooth muscle proliferation and the expression of excess matrix components: both processes are thought to contribute to reocclusion of vessels after coronary angioplasty.

•Transplantation.

NF- κ B is required for the induction of adhesion molecules (Eck et al., *supra*, K. O'Brien, et al., J. Clin. Invest. 92, 945-951 (1993)) that function in immune recognition and inflammatory responses. At least two potential modes of treatment are possible. In the first, transplanted organs are treated *ex vivo* with ribozymes or ribozyme expression vectors. Transient inhibition of NF- κ B in the transplanted endothelium may be sufficient to prevent transplant-associated vasculitis and may significantly modulate graft rejection. In the second, donor B cells are treated *ex vivo* with ribozymes or ribozyme expression vectors. Recipients would receive the treatment prior to transplant. Treatment of a recipient with B cells that do not express T cell co-stimulatory molecules (such as ICAM-1, VCAM-1, and/or B7 or B7-2) can induce antigen-specific anergy. Tolerance to the donor's histocompatibility antigens could result; potentially, any donor could be used for any transplantation procedure.

15 •Asthma.

Granulocyte macrophage colony stimulating factor (GM-CSF) is thought to play a major role in recruitment of eosinophils and other inflammatory cells during the late phase reaction to asthmatic trauma. Again, blocking the local induction of GM-CSF and other inflammatory mediators is likely to reduce the persistent inflammation observed in chronic asthmatics. Aerosol delivery of ribozymes or adenovirus ribozyme expression vectors is a feasible treatment.

•Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

Thus, ribozymes of the present invention that cleave *rel A* mRNA and thereby NF- κ B activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits NF- κ B

function is described above; available cellular and activity assays are number, reproducible, and accurate. Animal models for NF- κ B function (Kitajima, et al., *supra*) and for each of the suggested disease targets exist and can be used to optimize activity.

5 Example 4: TNF- α

Ribozymes that cleave the specific sites in TNF- α mRNA represent a novel therapeutic approach to inflammatory or autoimmune disorders.

- 10 Tumor necrosis factor- α (TNF- α) is a protein, secreted by activated leukocytes, that is a potent mediator of inflammatory reactions. Injection of TNF- α into experimental animals can simulate the symptoms of systemic and local inflammatory diseases such as septic shock or rheumatoid arthritis.

- 15 TNF- α was initially described as a factor secreted by activated macrophages which mediates the destruction of solid tumors in mice (Old, 1985 Science 230, 4225-4231). TNF- α subsequently was found to be identical to cachectin, an agent responsible for the weight loss and wasting syndrome associated with tumors and chronic infections (Beutler, et al., 1985 Nature 316, 552-554). The cDNA and the genomic locus for TNF- α have been cloned and found to be related to TNF- β (Shakhov et al., 1990
20 J. Exp. Med. 171, 35-47). Both TNF- α and TNF- β bind to the same receptors and have nearly identical biological activities. The two TNF receptors have been found on most cell types examined (Smith, et al., 1990 Science 248, 1019-1023). TNF- α secretion has been detected from monocytes/macrophages, CD4+ and CD8+ T-cells, B-cells, lymphokine
25 activated killer cells, neutrophils, astrocytes, endothelial cells, smooth muscle cells, as well as various non-hematopoietic tumor cell lines (for a review see Turetskaya et al., 1991 in Tumor Necrosis Factor. Structure, Function, and Mechanism of Action B. B. Aggarwal, J. Vilcek, Eds. Marcel Dekker, Inc., pp. 35-60). TNF- α is regulated transcriptionally and
30 translationally, and requires proteolytic processing at the plasma membrane in order to be secreted (Kriegler et al., 1988 Cell 53, 45-53). Once secreted, the serum half life of TNF- α is approximately 30 minutes. The tight regulation of TNF- α is important due to the extreme toxicity of this cytokine. Increasing evidence indicates that overproduction of TNF- α

during infections can lead to severe systemic toxicity and death (Tracey & Cerami, 1992 Am. J. Trop. Med. Hyg. 47, 2-7).

Antisense RNA and Hammerhead ribozymes have been used in an attempt to lower the expression level of TNF- α by targeting specified cleavage sites [Sioud et al., 1992 J. Mol. Biol. 223; 831; Sioud WO 94/10301; Kisich and co-workers, 1990 abstract (FASEB J. 4, A1860; 1991 slide presentation (J. Leukocyte Biol. sup. 2, 70); December, 1992 poster presentation at Anti-HIV Therapeutics Conference in San Diego, CA; and "Development of anti-TNF- α ribozymes for the control of TNF- α gene expression"- Kisich, Doctoral Dissertation, 1993 University of California, Davis] listing various TNF α targeted ribozymes.

Ribozymes of this invention block to some extent TNF- α expression and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of septic shock and rheumatoid arthritis. Ribozyme cleavage of TNF- α mRNA in these systems may prevent inflammatory cell function and alleviate disease symptoms.

The sequence of human and mouse TNF- α mRNA can be screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 23, 25, and 27 - 28. (All sequences are 5' to 3' in the tables.) While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme. (In Table 24, lower case letters indicate positions that are not conserved between the human and the mouse TNF- α sequences.)

The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 24, 26 - 28. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of hammerhead ribozymes listed in Tables 24 and 26 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any

sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in Tables 27 and 28 (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of

5 two base-paired stem structure can form. The sequences listed in Tables 24, 26 - 28 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables or AAV .

In a preferred embodiment of the invention, a transcription unit

10 expressing a ribozyme that cleaves TNF- α RNA is inserted into a plasmid DNA vector or an adenovirus DNA viral vector or AAV or alpha virus or retrovirus vectors. Viral vectors have been used to transfer genes to the intact vasculature or to joints of live animals (Willard et al., 1992 Circulation, 86, 1-473.; Nabel et al., 1990 Science, 249, 1285-1288) and

15 both vectors lead to transient gene expression. The adenovirus vector is delivered as recombinant adenoviral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant adenovirus particles are locally administered to the site of treatment, e.g., through the use of an

20 injection catheter, stent or infusion pump or are directly added to cells or tissues *ex vivo*.

In another preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves TNF- α RNA is inserted into a retrovirus vector for sustained expression of ribozyme(s).

25 By engineering ribozyme motifs we have designed several ribozymes directed against TNF- α mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave TNF- α target sequences *in vitro* is evaluated.

The ribozymes will be tested for function in cells by analyzing

30 bacterial lipopolysaccharide (LPS)-induced TNF- α expression levels. Ribozymes will be delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. TNF- α expression will be monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. TNF- α mRNA levels will be

35 assessed by Northern analysis, RNase protection, primer extension

analysis or quantitative RT-PCR. Ribozymes that block the induction of TNF- α activity and/or TNF- α mRNA by more than 90% will be identified.

RNA ribozymes and/or genes encoding them will be locally delivered to macrophages by intraperitoneal injection. After a period of ribozyme uptake, the peritoneal macrophages are harvested and induced *ex vivo* with LPS. The ribozymes that significantly reduce TNF- α secretion are selected. The TNF- α can also be induced after ribozyme treatment with fixed *Streptococcus* in the peritoneal cavity instead of *ex vivo*. In this fashion the ability of TNF- α ribozymes to block TNF- α secretion in a localized inflammatory response are evaluated. In addition, we will determine if the ribozymes can block an ongoing inflammatory response by delivering the TNF- α ribozymes after induction by the injection of fixed *Streptococcus*.

To examine the effect of anti-TNF- α ribozymes on systemic inflammation, the ribozymes are delivered by intravenous injection. The ability of the ribozymes to inhibit TNF- α secretion and lethal shock caused by systemic LPS administration are assessed. Similarly, TNF- α ribozymes can be introduced into the joints of mice with collagen-induced arthritis. Either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments can be used to supply ribozymes. One dose (or a few infrequent doses) of a stable anti-TNF- α ribozyme or a gene construct that constitutively expresses the ribozyme may abrogate tissue damage in these inflammatory diseases.

Macrophage isolation.

To produce responsive macrophages 1 ml of sterile fluid thioglycollate broth (Difco, Detroit, MI.) was injected i.p. into 6 week old female C57b1/6NCR mice 3 days before peritoneal lavage. Mice were maintained as specific pathogen free in autoclaved cages in a laminar flow hood and given sterilized water to minimize "spontaneous" activation of macrophages. The resulting peritoneal exudate cells (PEC) were obtained by lavage using Hanks balanced salt solution (HBSS) and were plated at 2.5×10^5 /well in 96 well plates (Costar, Cambridge, MA.) with Eagles minimal essential medium (EMEM) containing 10% heat inactivated fetal

bovine serum. After adhering for 2 hours the wells were washed to remove non-adherent cells. The resulting cultures were 97% macrophages as determined by morphology and staining for non-specific esterase.

Transfection of ribozymes into macrophages:

- 5 The ribozymes were diluted to 2X final concentration, mixed with an equal volume of 11nM lipofectamine (Life Technologies, Gaithersburg, MD.), and vortexed. 100 ml of lipid:ribozyme complex was then added directly to the cells, followed immediately by 10 ml fetal bovine serum. Three hours after ribozyme addition 100 ml of 1 mg/ml bacterial lipopolysaccharide (LPS) was added to each well to stimulate TNF production.
- 10

Quantitation of TNF- α in mouse macrophages:

- Supernatants were sampled at 0, 2, 4, 8, and 24 hours post LPS stimulation and stored at -70°C. Quantitation of TNF- α was done by a specific ELISA. ELISA plates were coated with rabbit anti-mouse TNF- α serum at 1:1000 dilution (Genzyme) followed by blocking with milk proteins and incubation with TNF- α containing supernatants. TNF- α was then detected using a murine TNF- α specific hamster monoclonal antibody (Genzyme). The ELISA was developed with goat anti-hamster IgG coupled to alkaline phosphatase.
- 15
- 20

Assessment of reagent toxicity:

- Following ribozyme/lipid treatment of macrophages and harvesting of supernatants viability of the cells was assessed by incubation of the cells with 5 mg/ml of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). This compound is reduced by the mitochondrial dihydrogenases, the activity of which correlates well with cell viability. After 12 hours the absorbance of reduced MTT is measured at 585 nm.
- 25

Uses

- The association between TNF- α and bacterial sepsis, rheumatoid arthritis, and autoimmune disease make TNF- α an attractive target for therapeutic intervention [Tracy & Cerami 1992 *supra*; Williams et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 9784-9788; Jacob, 1992 *J. Autoimmun.* 5 (Supp. A), 133-143].
- 30

Septic Shock

Septic shock is a complication of major surgery, bacterial infection, and polytrauma characterized by high fever, increased cardiac output, reduced blood pressure and a neutrophilic infiltrate into the lungs and other major organs. Current treatment options are limited to antibiotics to reduce the bacterial load and non-steroidal anti-inflammatories to reduce fever. Despite these treatments in the best intensive care settings, mortality from septic shock averages 50%, due primarily to multiple organ failure and disseminated vascular coagulation. Septic shock, with an incidence of 200,000 cases per year in the United States, is the major cause of death in intensive care units. In septic shock syndrome, tissue injury or bacterial products initiate massive immune activation, resulting in the secretion of pro-inflammatory cytokines which are not normally detected in the serum, such as TNF- α , interleukin-1 β (IL-1 β), γ -interferon (IFN- γ), interleukin-6 (IL-6), and interleukin-8 (IL-8). Other non-cytokine mediators such as leukotriene b₄, prostaglandin E₂, C3a and C3d also reach high levels (de Boer et al., 1992 Immunopharmacology 24, 135-148).

TNF- α is detected early in the course of septic shock in a large fraction of patients (de Boer et al., 1992 supra). In animal models, injection of TNF- α has been shown to induce shock-like symptoms similar to those induced by LPS injection (Beutler et al., 1985 Science 229, 869-871); in contrast, injection of IL-1 β , IL-6, or IL-8 does not induce shock. Injection of TNF- α also causes an elevation of IL-1 β , IL-6, IL-8, PgE₂, acute phase proteins, and TxA₂ in the serum of experimental animals (de Boer et al., 1992 supra). In animal models the lethal effects of LPS can be blocked by pre-administration of anti-TNF- α antibodies. The cumulative evidence indicates that TNF- α is a key player in the pathogenesis of septic shock, and therefore a good candidate for therapeutic intervention.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of the joints leading to bone destruction and loss of joint function. At the cellular level, autoreactive T- lymphocytes and monocytes are typically present, and the synoviocytes often have altered morphology and immunostaining patterns. RA joints have been shown to contain elevated levels of TNF- α , IL-1 α and IL-1 β , IL-6, GM-CSF, and TGF-

β (Abney et al., 1991 Imm. Rev. 119, 105-123), some or all of which may contribute to the pathological course of the disease.

Cells cultured from RA joints spontaneously secrete all of the pro-inflammatory cytokines detected *in vivo*. Addition of antisera against TNF- α to these cultures has been shown to reduce IL-1 α/β production by these cells to undetectable levels (Abney et al., 1991 Supra). Thus, TNF- α may directly induce the production of other cytokines in the RA joint. Addition of the anti-inflammatory cytokine, TGF- β , has no effect on cytokine secretion by RA cultures. Immunocytochemical studies of human RA surgical specimens clearly demonstrate the production of TNF- α , IL-1 α/β , and IL-6 from macrophages near the cartilage/pannus junction when the pannus is invading and overgrowing the cartilage (Chu et al., 1992 Br. J. Rheumatology 31, 653-661). GM-CSF was shown to be produced mainly by vascular endothelium in these samples. Both TNF- α and TGF- β have been shown to be fibroblast growth factors, and may contribute to the accumulation of scar tissue in the RA joint. TNF- α has also been shown to increase osteoclast activity and bone resorption, and may have a role in the bone erosion commonly found in the RA joint (Cooper et al., 1992 Clin. Exp. Immunol. 89, 244-250).

Elimination of TNF- α from the rheumatic joint would be predicted to reduce overall inflammation by reducing induction of MHC class II, IL-1 α/β , IL-6, and GM-CSF, and reducing T-cell activation. Osteoclast activity might also fall, reducing the rate of bone erosion at the joint. Finally, elimination of TNF- α would be expected to reduce accumulation of scar tissue within the joint by removal of a fibroblast growth factor.

Treatment with an anti-TNF- α antibody reduces joint swelling and the histological severity of collagen-induced arthritis in mice (Williams et al., 1992 Proc. Natl. Acad. Sci. USA 89, 9784-9788). In addition, a study of RA patients who have received i.v. infusions of anti-TNF- α monoclonal antibody reports a reduction in the number and severity of inflamed joints after treatment. The benefit of monoclonal antibody treatment in the long term may be limited by the expense and immunogenicity of the antibody.

Psoriasis

Psoriasis is an inflammatory disorder of the skin characterized by keratinocyte hyperproliferation and immune cell infiltrate (Kupper, 1990 J.

Clin. Invest. 86, 1783-1789). It is a fairly common condition, affecting 1.5-2.0% of the population. The disorder ranges in severity from mild, with small flaky patches of skin, to severe, involving inflammation of the entire epidermis. The cellular infiltrate of psoriasis includes T-lymphocytes, 5 neutrophils, macrophages, and dermal dendrocytes. The majority of T-lymphocytes are activated CD4⁺ cells of the T_H-1 phenotype, although some CD8⁺ and CD4⁺/CD8⁺ are also present. B lymphocytes are typically not found in abundance in psoriatic plaques.

Numerous hypotheses have been offered as to the proximal cause of psoriasis including auto-antibodies and auto-reactive T-cells, 10 overproduction of growth factors, and genetic predisposition. Although there is evidence to support the involvement of each of these factors in psoriasis, they are neither mutually exclusive nor are any of them necessary and sufficient for the pathogenesis of psoriasis (Reeves, 1991 15 Semin. Dermatol. 10, 217).

The role of cytokines in the pathogenesis of psoriasis has been investigated. Among those cytokines found to be abnormally expressed were TGF- α , IL-1 α , IL-1 β , IL-1 α , IL-6, IL-8, IFN- γ , and TNF- α . In addition to abnormal cytokine production, elevated expression of ICAM-1, ELAM-1, 20 and VCAM has been observed (Reeves, 1991 supra). This cytokine profile is similar to that of normal wound healing, with the notable exception that cytokine levels subside upon healing. Keratinocytes themselves have recently been shown to be capable of secreting EGF, TGF- α , IL-6, and TNF- α , which could increase proliferation in an autocrine fashion (Oxholm 25 et al., 1991 APMIS 99, 58-64).

Nickoloff et al., 1993 (J Dermatol Sci. 6, 127-33) have proposed the following model for the initiation and maintenance of the psoriatic plaque:

Tissue damage induces the wound healing response in the skin. Keratinocytes secrete IL-1 α , IL-1 β , IL-6, IL-8, TNF- α . These factors 30 activate the endothelium of dermal capillaries, recruiting PMNs, macrophages, and T-cells into the wound site.

Dermal dendrocytes near the dermal/epidermal junction remain activated when they should return to a quiescent state, and subsequently secrete cytokines including TNF- α , IL-6, and IL-8. Cytokine expression, in

turn, maintains the activated state of the endothelium, allowing extravasation of additional immunocytes, and the activated state of the keratinocytes which secrete TGF- α and IL-8. Keratinocyte IL-8 recruits immunocytes from the dermis into the epidermis. During passage through
5 the dermis, T-cells encounter the activated dermal dendrocytes which efficiently activate the T_H-1 phenotype. The activated T-cells continue to migrate into the epidermis, where they are stimulated by keratinocyte-expressed ICAM-1 and MHC class II. IFN- γ secreted by the T-cells synergizes with the TNF- α from dermal dendrocytes to increase
10 keratinocyte proliferation and the levels of TGF- α , IL-8, and IL-6 production. IFN- γ also feeds back to the dermal dendrocyte, maintaining the activated phenotype and the inflammatory cycle.

Elevated serum titres of IL-6 increases synthesis of acute phase proteins including complement factors by the liver, and antibody production
15 by plasma cells. Increased complement and antibody levels increases the probability of autoimmune reactions.

Maintenance of the psoriatic plaque requires continued expression of all of these processes, but attractive points of therapeutic intervention are TNF- α expression by the dermal dendrocyte to maintain activated
20 endothelium and keratinocytes, and IFN- γ expression by T-cells to maintain activated dermal dendrocytes.

There are 3 million patients in the United States afflicted with psoriasis. The available treatments for psoriasis are corticosteroids. The most widely prescribed are TEMOVATE (clobetasol propionate), LIDEX
25 (fluocinonide), DIPROLENE (betamethasone propionate), PSORCON (diflorasone diacetate) and TRIAMCINOLONE formulated for topical application. The mechanism of action of corticosteroids is multifactorial. This is a palliative therapy because the underlying cause of the disease remains, and upon discontinuation of the treatment the disease returns.
30 Discontinuation of treatment is often prompted by the appearance of adverse effects such as atrophy, telangiectasias and purpura. Corticosteroids are not recommended for prolonged treatments or when treatment of large and/or inflamed areas is required. Alternative treatments include retinoids, such as etretinate, which has been approved for
35 treatment of severe, refractory psoriasis. Alternative retinoid-based treatments are in advanced clinical trials. Retinoids act by converting

- keratinocytes to a differentiated state and restoration of normal skin development. Immunosuppressive drugs such as cyclosporine are also in the advanced stages of clinical trials. Due to the nonspecific mechanism of action of corticosteroids, retinoids and immunosuppressives, these
- 5 treatments exhibit severe side effects and should not be used for extended periods of time unless the condition is life-threatening or disabling. There is a need for a less toxic, effective therapeutic agent in psoriatic patients.

HIV and AIDS

- The human immunodeficiency virus (HIV) causes several
- 10 fundamental changes in the human immune system from the time of infection until the development of full-blown acquired immunodeficiency syndrome (AIDS). These changes include a shift in the ratio of CD4+ to CD8+ T-cells, sustained elevation of IL-4 levels, episodic elevation of TNF- α and TNF- β levels, hypergammaglobulinemia, and lymphoma/leukemia
- 15 (Rosenberg & Fauci, 1990 Immun. Today 11, 176; Weiss 1993 Science 260, 1273). Many patients experience a unique tumor, Kaposi's sarcoma and/or unusual opportunistic infections (e.g. *Pneumocystis carinii*, cytomegalovirus, herpesviruses, hepatitis viruses, papilloma viruses, and tuberculosis). The immunological dysfunction of individuals with AIDS
- 20 suggests that some of the pathology may be due to cytokine dysregulation.

- Levels of serum TNF- α and IL-6 are often found to be elevated in AIDS patients (Weiss, 1993 supra). In tissue culture, HIV infection of monocytes isolated from healthy individuals stimulates secretion of both TNF- α and IL-6. This response has been reproduced using purified gp120,
- 25 the viral coat protein responsible for binding to CD-4 (Buonaguro et al., 1992 J. Virol. 66, 7159). It has also been demonstrated that the viral gene regulator, Tat, can directly induce TNF transcription. The ability of HIV to directly stimulate secretion of TNF- α and IL-6 may be an adaptive mechanism of the virus. TNF- α has been shown to upregulate transcription
- 30 of the LTR of HIV, increasing the number of HIV-specific transcripts in infected cells. IL-6 enhances HIV production, but at a post-transcriptional level, apparently increasing the efficiency with which HIV transcripts are translated into protein. Thus, stimulation of TNF- α secretion by the HIV virus may promote infection of neighboring CD4+ cells both by enhancing
- 35 virus production from latently infected cells and by driving replication of the virus in newly infected cells.

The role of TNF- α in HIV replication has been well established in tissue culture models of infection (Sher et al., 1992 Immun. Rev. 127, 183), suggesting that the mutual induction of HIV replication and TNF- α replication may create positive feedback *in vivo*. However, evidence for the presence of such positive feedback in infected patients is not abundant. TNF- α levels are found to be elevated in some, but not all patients tested. Children with AIDS who were given zidovudine had reduced levels of TNF- α compared to those not given zidovudine (Cremoni et al., 1993 AIDS 7, 128). This correlation lends support to the hypothesis that reduced viral replication is physiologically linked to TNF- α levels. Furthermore, recently it has been shown that the polyclonal B cell activation associated with HIV infection is due to membrane-bound TNF- α . Thus, levels of secreted TNF- α may not accurately reflect the contribution of this cytokine to AIDS pathogenesis.

Chronic elevation of TNF- α has been shown to result in cachexia (Tracey et al., 1992 Am. J. Trop. Med. Hyg. 47, 2-7), increased autoimmune disease (Jacob, 1992 supra), lethargy, and immune suppression in animal models (Aderka et al., 1992 Isr. J. Med. Sci. 28, 126-130). The cachexia associated with AIDS may be associated with chronically elevated TNF- α frequently observed in AIDS patients. Similarly, TNF- α can stimulate the proliferation of spindle cells isolated from Kaposi's sarcoma lesions of AIDS patients (Barillari et al., 1992 J. Immunol 149, 3727).

A therapeutic agent that inhibits cytokine gene expression, inhibits adhesion molecule expression, and mimics the anti-inflammatory effects of glucocorticoids (without inducing steroid-responsive genes) is ideal for the treatment of inflammatory and autoimmune disorders. Disease targets for such a drug are numerous. Target indications and the delivery options each entails are summarized below. In all cases, because of the potential immunosuppressive properties of a ribozyme that cleaves the specified sites in TNF- α mRNA, uses are limited to local delivery, acute indications, or *ex vivo* treatment.

•Septic shock.

Exogenous delivery of ribozymes to macrophages can be achieved by intraperitoneal or intravenous injections. Ribozymes will be delivered by incorporation into liposomes or by complexing with cationic lipids.

•Rheumatoid arthritis (RA).

- 5 Due to the chronic nature of RA, a gene therapy approach is logical. Delivery of a ribozyme to inflamed joints is mediated by adenovirus, retrovirus, or adeno-associated virus vectors. For instance, the appropriate adenovirus vector can be administered by direct injection into the synovium: high efficiency of gene transfer and expression for several
- 10 months would be expected (B.J. Roessler, E.D. Allen, J.M. Wilson, J.W. Hartman, B. L. Davidson, J. Clin. Invest. 92, 1085-1092 (1993)). It is unlikely that the course of the disease could be reversed by the transient, local administration of an anti-inflammatory agent. Multiple administrations may be necessary. Retrovirus and adeno-associated virus
- 15 vectors would lead to permanent gene transfer and expression in the joint. However, permanent expression of a potent anti-inflammatory agent may lead to local immune deficiency.

•Psoriasis

- 20 The psoriatic plaque is a particularly good candidate for ribozyme or vector delivery. The stratum comeum of the plaque is thinned, providing access to the proliferating keratinocytes. T-cells and dermal dendrocytes can be efficiently targeted by trans-epidermal diffusion .

- Organ culture systems for biopsy specimens of psoriatic and normal skin are described in current literature (Nickoloff et al., 1993 Supra).
- 25 Primary human keratinocytes are easily obtained and will be grown into epidermal sheets in tissue culture. In addition to these tissue culture models, the flaky skin mouse develops psoriatic skin in response to UV light. This model would allow demonstration of animal efficacy for ribozyme treatments of psoriasis.

- 30 •Gene Therapy.

Immune responses limit the efficacy of many gene transfer techniques. Cells transfected with retrovirus vectors have short lifetimes in immune competent individuals. The length of expression of adenovirus

vectors in terminally differentiated cells is longer in neonatal or immune-compromised animals. Insertion of a small ribozyme expression cassette that modulates inflammatory and immune responses into existing adenovirus or retrovirus constructs will greatly enhance their potential.

- 5 Thus, ribozymes of the present invention that cleave TNF- α mRNA and thereby TNF- α activity have many potential therapeutic uses, and there are reasonable modes of delivering the ribozymes in a number of the possible indications. Development of an effective ribozyme that inhibits TNF- α function is described above; available cellular and activity assays
10 are number, reproducible, and accurate. Animal models for TNF- α function and for each of the suggested disease targets exist and can be used to optimize activity.

Example 5: p210^{bcr-abl}

- Chronic myelogenous leukemia exhibits a characteristic disease
15 course, presenting initially as a chronic granulocytic hyperplasia, and invariably evolving into an acute leukemia which is caused by the clonal expansion of a cell with a less differentiated phenotype (i.e., the blast crisis stage of the disease). CML is an unstable disease which ultimately progresses to a terminal stage which resembles acute leukemia. This
20 lethal disease affects approximately 16,000 patients a year. Chemotherapeutic agents such as hydroxyurea or busulfan can reduce the leukemic burden but do not impact the life expectancy of the patient (e.g., approximately 4 years). Consequently, CML patients are candidates for bone marrow transplantation (BMT) therapy. However, for those patients
25 which survive BMT, disease recurrence remains a major obstacle (Apperley et al., 1988 Br. J. Haematol. 69, 239).

- The Philadelphia (Ph) chromosome which results from the translocation of the *abl* oncogene from chromosome 9 to the *bcr* gene on chromosome 22 is found in greater than 95% of CML patients and in 10-
30 25% of all cases of acute lymphoblastic leukemia [(ALL); Fourth International Workshop on Chromosomes in Leukemia 1982, Cancer Genet. Cytogenet. 11, 316]. In virtually all Ph-positive CMLs and approximately 50% of the Ph-positive ALLs, the leukemic cells express *bcr-abl* fusion mRNAs in which exon 2 (b2-a2 junction) or exon 3 (b3-a2
35 junction) from the major breakpoint cluster region of the *bcr* gene is spliced

- to exon 2 of the *abl* gene. Heisterkamp et al., 1985 Nature 315, 758; Shtivelman et al., 1987, Blood 69, 971). In the remaining cases of Ph-positive ALL, the first exon of the *bcr* gene is spliced to exon 2 of the *abl* gene (Hooberman et al., 1989 Proc. Nat. Acad. Sci. USA 86, 4259; 5 Heisterkamp et al., 1988 Nucleic Acids Res. 16, 10069).

- The b3-a2 and b2-a2 fusion mRNAs encode 210 kd bcr-abl fusion proteins which exhibit oncogenic activity (Daley et al., 1990 Science 247, 824; Heisterkamp et al., 1990 Nature 344, 251). The importance of the bcr-abl fusion protein (p210^{bcr-abl}) in the evolution and maintenance of the 10 leukemic phenotype in human disease has been demonstrated using antisense oligonucleotide inhibition of p210^{bcr-abl} expression. These inhibitory molecules have been shown to inhibit the in vitro proliferation of leukemic cells in bone marrow from CML patients. Szczylik et al., 1991 Science 253, 562).

- 15 Reddy, U.S. Patent 5,246,921 (hereby incorporated by reference herein) describes use of ribozymes as therapeutic agents for leukemias, such as chronic myelogenous leukemia (CML) by targeting the specific junction region of *bcr-abl* fusion transcripts. It indicates causing cleavage by a ribozyme at or near the breakpoint of such a hybrid chromosome, 20 specifically it includes cleavage at the sequence GUX, where X is A, U or G. The one example presented is to cleave the sequence 5' AGC AG AGUU (cleavage site) CAA AAGCCCU-3'.

- Scanlon WO 91/18625, WO 91/18624, and WO 91/18913 and Snyder et al., WO93/03141 and WO94/13793 describe a ribozyme effective 25 to cleave oncogenic variants of H-ras RNA. This ribozyme is said to inhibit H-ras expression in response to external stimuli.

- The invention features use of ribozymes to inhibit the development or expression of a transformed phenotype in man and other animals by modulating expression of a gene that contributes to the expression of CML. 30 Cleavage of targeted mRNAs expressed in pre-neoplastic and transformed cells elicits inhibition of the transformed state.

The invention can be used to treat cancer or pre-neoplastic conditions. Two preferred administration protocols can be used, either in vivo administration to reduce the tumor burden, or ex vivo treatment to

eradicate transformed cells from tissues such as bone marrow prior to reimplantation.

This invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of CML. The mRNA targets are present in the 425 nucleotides surrounding the fusion sites of the *bcr* and *abl* sequences in the b2-a2 and b3-a2 recombinant mRNAs. Other sequences in the 5' portion of the *bcr* mRNA or the 3' portion of the *abl* mRNA may also be targeted for ribozyme cleavage. Cleavage at any of these sites in the fusion mRNA molecules will result in inhibition of translation of the fusion protein in treated cells.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of CML. Such enzymatic RNA molecules can be delivered exogenously or endogenously to afflicted cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The smallest ribozyme delivered for any type of treatment reported to date (by Rossi et al., 1992 *supra*) is an *in vitro* transcript having a length of 142 nucleotides. Synthesis of ribozymes greater than 100 nucleotides in length is very difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. Delivery of ribozymes by expression vectors is primarily feasible using only *ex vivo* treatments. This limits the utility of this approach. In this invention, an alternative approach uses smaller ribozyme motifs and exogenous delivery. The simple structure of these molecules also increases the ability of the ribozyme to invade targeted regions of the mRNA structure. Thus, unlike the situation when the hammerhead structure is included within longer transcripts, there are no non-ribozyme flanking sequences to interfere with correct folding of the ribozyme structure, as well as complementary binding of the ribozyme to the mRNA target.

The enzymatic RNA molecules of this invention can be used to treat human CML or precancerous conditions. Affected animals can be treated at the time of cancer detection or in a prophylactic manner. This timing of treatment will reduce the number of affected cells and disable cellular

replication. This is possible because the ribozymes are designed to disable those structures required for successful cellular proliferation.

Ribozymes of this invention block to some extent p210^{bcr-abl} expression and can be used to treat disease or diagnose such disease.

- 5 Ribozymes will be delivered to cells in culture and to tissues in animal models of CML. Ribozyme cleavage of *bcr-abl* mRNA in these systems may prevent or alleviate disease symptoms or conditions.

- 10 The sequence of human *bcr-abl* mRNA can be screened for accessible sites using a computer folding algorithm. Regions of the mRNA that did not form secondary folding structures and that contain potential hammerhead or hairpin ribozyme cleavage sites can be identified. These sites are shown in Table 29 (All sequences are 5' to 3' in the tables). The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

- 15 The sequences of the chemically synthesized ribozymes most useful in this study are shown in Table 30. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of
20 hammerhead ribozymes listed in Table 30 (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables 30 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the
25 ribozymes described specifically in the Tables.

By engineering ribozyme motifs we have designed several ribozymes directed against *bcr-abl* mRNA sequences. These have been synthesized with modifications that improve their nuclease resistance as described above. These ribozymes cleave *bcr-abl* target sequences *in vitro*.

- 30 The ribozymes are tested for function *in vivo* by exogenous delivery to cells expressing *bcr-abl*. Ribozymes are delivered by incorporation into liposomes, by complexing with cationic lipids, by microinjection, or by expression from DNA vectors. Expression of *bcr-abl* is monitored by ELISA, by indirect immunofluorescence, and/or by FACS analysis. Levels of

bcr-abl mRNA are assessed by Northern analysis, RNase protection, by primer extension analysis or by quantitative RT-PCR techniques. Ribozymes that block the induction of p210^{*bcr-abl*} protein and mRNA by more than 20% are identified.

5 Example 6: RSV

This invention relates to the use of ribozymes as inhibitors of respiratory syncytial virus (RSV) production, and in particular, the inhibition of RSV replication.

RSV is a member of the virus family paramyxoviridae and is classified under the genus *Pneumovirus* (for a review see McIntosh and Chanock, 1990 in Virology ed. B.N. Fields, pp. 1045, Raven Press Ltd. NY). The infectious virus particle is composed of a nucleocapsid enclosed within an envelope. The nucleocapsid is composed of a linear negative single-stranded non-segmented RNA associated with repeating subunits of capsid proteins to form a compact structure and thereby protect the RNA from nuclease degradation. The entire nucleocapsid is enclosed by the envelope. The size of the virus particle ranges from 150 - 300 nm in diameter. The complete life cycle of RSV takes place in the cytoplasm of infected cells and the nucleocapsid never reaches the nuclear compartment (Hall, 1990 in Principles and Practice of Infectious Diseases ed. Mandell et al., Churchill Livingstone, NY).

The RSV genome encodes ten viral proteins essential for viral production. RSV protein products include two structural glycoproteins (G and F) found in the envelope spikes, two matrix proteins [M and M2 (22K)] found in the inner membrane, three proteins localized in the nucleocapsid (N, P and L), one protein that is present on the surface of the infected cell (SH), and two nonstructural proteins [NS1 (1C) and NS2 (1B)] found only in the infected cell. The mRNAs for the 10 RSV proteins have similar 5' and 3' ends. UV-inactivation studies suggest that a single promoter is used with multiple transcription initiation sites (Barik *et al.*, 1992 J. Virol. 66, 6813). The order of transcription corresponding to the protein assignment on the genomic RNA is 1C, 1B, N, P, M, SH, G, F, 22K and L genes (Huang *et al.*, 1985 Virus Res. 2, 157) and transcript abundance corresponds to the order of gene assignment (for example the 1C and 1B mRNAs are much more abundant than the L mRNA. Synthesis of viral message begins

immediately after RSV infection of cells and reaches a maximum at 14 hours post-infection (McIntosh and Chanock, *supra*).

There are two antigenic subgroups of RSV, A and B, which can circulate simultaneously in the community in varying proportions in different years (McIntosh and Chanock, *supra*). Subgroup A usually predominates. Within the two subgroups there are numerous strains. By the limited sequence analysis available it seems that homology at the nucleotide level is more complete within than between subgroups, although sequence divergence has been noted within subgroups as well. Antigenic determinates result primarily from both surface glycoproteins, F and G. For F, at least half of the neutralization epitopes have been stably maintained over a period of 30 years. For G however, A and B subgroups may be related antigenically by as little as a few percent. On the nucleotide level, however, the majority of the divergence in the coding region of G is found in the sequence for the extracellular domain (Johnson et al., 1987, *Proc. Natl. Acad. Sci. USA* 84, 5625).

Respiratory Syncytial Virus (RSV) is the major cause of lower respiratory tract illness during infancy and childhood (Hall, *supra*) and as such is associated with an estimated 90,000 hospitalizations and 4500 deaths in the United States alone (Update: respiratory syncytial virus activity - United States, 1993, *Mmwr Morb Mortal Wkly Rep*, 42, 971). Infection with RSV generally outranks all other microbial agents leading to both pneumonia and bronchitis. While primarily affecting children under two years of age, immunity is not complete and reinfection of older children and adults, especially hospital care givers (McIntosh and Chanock, *supra*), is not uncommon. Immunocompromised patients are severely affected and RSV infection is a major complication for patients undergoing bone marrow transplantation.

Uneventful RSV respiratory disease resembles a common cold and recovery is in 7 to 12 days. Initial symptoms (rhinorrhea, nasal congestion, slight fever, etc.) are followed in 1 to 3 days by lower respiratory tract signs of infection that include a cough and wheezing. In severe cases, these mild symptoms quickly progress to tachypnea, cyanosis, and listlessness and hospitalization is required. In infants with underlying cardiac or respiratory disease, the progression of symptoms is especially rapid and can lead to respiratory failure by the second or third day of illness. With

modern intensive care however, overall mortality is usually less than 5% of hospitalized patients (McIntosh and Chanock, *supra*).

At present, neither an efficient vaccine nor a specific antiviral agent is available. An immune response to the viral surface glycoproteins can
5 provide resistance to RSV in a number of experimental animals, and a subunit vaccine has been shown to be effective for up to 6 months in children previously hospitalized with an RSV infection (Tristram *et al.*, 1993, J. Infect. Dis. 167, 191). An attenuated bovine RSV vaccine has also been shown to be effective in calves for a similar length of time (Kubota *et al.*,
10 1992 J. Vet. Med. Sci. 54, 957). Previously however, a formalin-inactivated RSV vaccine was implicated in greater frequency of severe disease in subsequent natural infections with RSV (Connors *et al.*, 1992 J. Virol. 66, 7444).

The current treatment for RSV infection requiring hospitalization is the
15 use of aerosolized ribavirin, a guanosine analog [Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY.]. Ribavirin therapy is associated with a decrease in the severity of the symptoms, improved arterial oxygen and a decrease in the amount of viral shedding at the end of the treatment
20 period. It is not certain, however, whether ribavirin therapy actually shortens the patients' hospital stay or diminishes the need for supportive therapies (McIntosh and Chanock, *supra*). The benefits of ribavirin therapy are especially clear for high risk infants, those with the most serious symptoms or for patients with underlying bronchopulmonary or cardiac
25 disease. Inhibition of the viral polymerase complex is supported as the main mechanism for inhibition of RSV by ribavirin, since viral but not cellular polypeptide synthesis is inhibited by ribavirin in RSV-infected cells (Antiviral Agents and Viral Diseases of Man, 3rd edition. 1990. (eds. G.J. Galasso, R.J. Whitley, and T.C. Merigan) Raven Press Ltd., NY). Since
30 ribavirin is at least partially effective against RSV infection when delivered by aerosolization, it can be assumed that the target cells are at or near the epithelial surface. In this regard, RSV antigen had not spread any deeper than the superficial layers of the respiratory epithelium in autopsy studies of fatal pneumonia (McIntosh and Chanock, *supra*).

35 Jennings *et al.*, WO 94/13688 indicates that targets for specific types of ribozymes include respiratory syncytical virus.

The invention features novel enzymatic RNA molecules, or ribozymes, and methods for their use for inhibiting production of respiratory syncytial virus (RSV). Such ribozymes can be used in a method for treatment of diseases caused by these related viruses in man and other animals. The invention also features cleavage of the genomic RNA and mRNA of these viruses by use of ribozymes. In particular, the ribozyme molecules described are targeted to the *NS1 (1C)*, *NS2 (1B)* and *N* viral genes. These genes are known in the art (for a review see McIntosh and Chanock, 1990 *supra*).

10 Ribozymes that cleave the specified sites in RSV mRNAs represent a novel therapeutic approach to respiratory disorders. Applicant indicates that ribozymes are able to inhibit the activity of RSV and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described
15 that other ribozymes that cleave these sites in RSV mRNAs encoding 1C, 1B and N proteins may be readily designed and are within the invention. Also, those of ordinary skill in the art, will find that it is clear from the examples described that ribozymes cleaving other mRNAs encoded by RSV (*P*, *M*, *SH*, *G*, *F*, 22K and *L*) and the genomic RNA may be readily
20 designed and are within the invention.

In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables 31, 33, 35, 37 and 38. Examples of such ribozymes are shown in Tables 32, 34, 36-38. Examples of such ribozymes consist essentially of sequences defined in these
25 Tables. By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

30 Ribozymes of this invention block to some extent RSV production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture and to cells or tissues in animal models of respiratory disorders. Ribozyme cleavage of RSV encoded mRNAs or the genomic RNA in these systems may alleviate disease symptoms.

While all ten RSV encoded proteins (1C, 1B, N, P, M, SH, 22K, F, G, and L) are essential for viral life cycle and are all potential targets for ribozyme cleavage, certain proteins (mRNAs) are more favorable for ribozyme targeting than the others. For example RSV encoded proteins 1C, 1B, SH and 22K are not found in other members of the family paramyxoviridae and appear to be unique to RSV. In contrast the ectodomain of the G protein and the signal sequence of the F protein show significant sequence divergence at the nucleotide level among various RSV sub-groups (Johnson *et al.*, 1987 *supra*). RSV proteins 1C, 1B and N are highly conserved among various subtypes at both the nucleotide and amino acid levels. Also, 1C, 1B and N are the most abundant of all RSV proteins.

The sequence of human RSV mRNAs encoding 1C, 1B and N proteins are screened for accessible sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables 31, 33, 34, 37 and 38 (All sequences are 5' to 3' in the tables.) The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe *et al.*, 1990 Nucleic Acids Res., 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G5 and a U for A14 (numbering from Hertel *et al.*, 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). Hairpin ribozymes are also synthesized from DNA templates using bacteriophage T7 RNA polymerase (Milligan and Uhlenbeck, 1989, *Methods Enzymol.* 180, 51). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant

groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-o-methyl, 2'-H (for a review see Usman and Cedergren, 1992 *TIBS* 17, 34). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography and are resuspended in water.

5 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables 32, 34, 36, 37 and 38. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the binding arms) is altered to affect activity. For example, stem-loop II sequence of
10 hammerhead ribozymes listed in Tables 32 and 34(5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion, and/or insertion) to contain any sequences provided a minimum of two base-paired stem structure can form. Similarly, stem-loop IV sequence of hairpin ribozymes listed in
15 Tables 37 and 38 (5'-CACGUUGUG-3') can be altered (substitution, deletion, and/or insertion) to contain any sequence, provided a minimum of two base-paired stem structure can form. The sequences listed in Tables 32, 34, 36, 37 and 38 may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

20 By engineering ribozyme motifs we have designed several ribozymes directed against RSV encoded mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in vitro* is evaluated.

25 Numerous, common cell lines can be infected with RSV for experimental purposes. These include *HeLa*, *Vero* and several primary epithelial cell lines. A cotton rat animal model of experimental human RSV infection is also available, and the bovine RSV is quite homologous to the human viruses. Rapid clinical diagnosis is through the use of kits designed for the immunofluorescence staining of RSV-infected cells or an ELISA
30 assay, both of which are adaptable for experimental study. RSV encoded mRNA levels will be assessed by Northern analysis, RNase protection, primer extension analysis or quantitative RT-PCR. Ribozymes that block the induction of RSV activity and/or 1C, 1B and N protein encoding mRNAs by more than 90% will be identified.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Draper et al., PCT WO93/23569. The details will not be repeated here, but include altering the length of the ribozyme binding arms or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 Nature 344, 565; Pieken et al., 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162, as well as Jennings et al., WO 94/13688, which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.), modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, et al., PCT WO94/02595, incorporated by reference herein, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. The RNA/vehicle combination is locally delivered by direct injection or by use of a catheter, infusion pump or stent. Alternative routes of delivery include, but are not limited to, intravenous injection, intramuscular injection, subcutaneous injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan, et al., supra and Draper, et al., supra which have been incorporated by reference herein.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given

pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells

5 (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet

10 et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U S A, 90, 6340-4; L'Huillier et al., 1992 EMBO J., 11, 4411-8; Lisiewicz et al., 1993 Proc. Natl. Acad. Sci. U. S. A., 90, 8000-4). The above ribozyme transcription units can be

15 incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral, or alpha virus vectors).

In a preferred embodiment of the invention, a transcription unit

20 expressing a ribozyme that cleaves target RNA is inserted into a plasmid DNA vector, a retrovirus DNA viral vector, an adenovirus DNA viral vector or an adeno-associated virus vector or alpha virus vector. These and other vectors have been used to transfer genes to live animals (for a review see Friedman, 1989 Science 244, 1275-1281; Roemer and Friedman, 1992

25 Eur. J. Biochem., 208, 211-225) and leads to transient or stable gene expression. The vectors are delivered as recombinant viral particles. DNA may be delivered alone or complexed with vehicles (as described for RNA above). The DNA, DNA/vehicle complexes, or the recombinant virus particles are locally administered to the site of treatment, e.g., through the

30 use of a catheter, stent or infusion pump.

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells. The close relationship between ribozyme activity and the structure of the target RNA

35 allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By

using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNA associated with ICAM-1, relA, TNF- α , p210, bcr-abl or RSV related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., ICAM-1, rel A, TNF- α , p210bcr-abl or RSV) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will

decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

II. Chemical Synthesis Of Ribozymes

- 5 There follows the chemical synthesis, deprotection, and purification of RNA, enzymatic RNA or modified RNA molecules in greater than milligram quantities with high biological activity. Applicant has determined that the synthesis of enzymatically active RNA in high yield and quantity is dependent upon certain critical steps used during its preparation.
- 10 Specifically, it is important that the RNA phosphoramidites are coupled efficiently in terms of both yield and time, that correct exocyclic amino protecting groups be used, that the appropriate conditions for the removal of the exocyclic amino protecting groups and the alkylsilyl protecting groups on the 2'-hydroxyl are used, and that the correct work-up and
- 15 purification procedure of the resulting ribozyme be used.

- To obtain a correct synthesis in terms of yield and biological activity of a large RNA molecule (*i.e.*, about 30 to 40 nucleotide bases), the protection of the amino functions of the bases requires either amide or substituted amide protecting groups, which must be, on the one hand, stable enough
- 20 to survive the conditions of synthesis, and on the other hand, removable at the end of the synthesis. These requirements are met by the amide protecting groups shown in Figure 8, in particular, benzoyl for adenosine, isobutyryl or benzoyl for cytidine, and isobutyryl for guanosine, which may be removed at the end of the synthesis by incubating the RNA in NH_3/EtOH
- 25 (ethanolic ammonia) for 20 h at 65 °C. In the case of the phenoxyacetyl type protecting groups shown in Figure 8 on guanosine and adenosine and acetyl protecting groups on cytidine, an incubation in ethanolic ammonia for 4 h at 65 °C is used to obtain complete removal of these protecting groups. Removal of the alkylsilyl 2'-hydroxyl protecting groups
- 30 can be accomplished using a tetrahydrofuran solution of TBAF at room temperature for 8-24 h.

- The most quantitative procedure for recovering the fully deprotected RNA molecule is by either ethanol precipitation, or an anion exchange cartridge desalting, as described in Scaringe *et al. Nucleic Acids Res.*
- 35 1990, 18, 5433-5341. The purification of the long RNA sequences may be

accomplished by a two-step chromatographic procedure in which the molecule is first purified on a reverse phase column with either the trityl group at the 5' position on or off. This purification is accomplished using an acetonitrile gradient with triethylammonium or bicarbonate salts as the aqueous phase. In the case of the trityl on purification, the trityl group may be removed by the addition of an acid and drying of the partially purified RNA molecule. The final purification is carried out on an anion exchange column, using alkali metal perchlorate salt gradients to elute the fully purified RNA molecule as the appropriate metal salts, e.g. Na⁺, Li⁺ etc. A final de-salting step on a small reverse-phase cartridge completes the purification procedure. Applicant has found that such a procedure not only fails to adversely affect activity of a ribozyme, but may improve its activity to cleave target RNA molecules.

Applicant has also determined that significant (see Tables 39-41) improvements in the yield of desired full length product (FLP) can be obtained by:

1. Using 5-S-alkyltetrazole at a delivered or effective concentration of 0.25-0.5 M or 0.15-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. (By delivered is meant that the actual amount of chemical in the reaction mix is known. This is possible for large scale synthesis since the reaction vessel is of size sufficient to allow such manipulations. The term effective means that available amount of chemical actually provided to the reaction mixture that is able to react with the other reagents present in the mixture. Those skilled in the art will recognize the meaning of these terms from the examples provided herein.) The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m. Alkyl, as used herein, refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to

- 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an
- 5 unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the
- 10 substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.

- Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated π electron
- 15 system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as
- 20 described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur,
- 25 and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

- 30 2. Using 5-S-alkyltetrazole at an effective, or final, concentration of 0.1-0.35 M for the activation of the RNA (or analogue) amidite during the coupling step. The time for this step is shortened from 10-15 m, *vide supra*, to 5-10 m.

3. Using alkylamine (MA, where alkyl is preferably methyl, ethyl,
- 35 propyl or butyl) or NH₄OH/alkylamine (AMA, with the same preferred alkyl groups as noted for MA) @ 65 °C for 10-15 m to remove the exocyclic

amino protecting groups (vs 4-20 h @ 55-65 °C using NH₄OH/EtOH or NH₃/EtOH, *vide supra*). Other alkylamines, e.g. ethylamine, propylamine, butylamine *etc.* may also be used.

4. Using anhydrous triethylamine•hydrogen fluoride (aHF•TEA)
5 @ 65 °C for 0.5-1.5 h to remove the 2'-hydroxyl alkylsilyl protecting group (vs 8 - 24 h using TBAF, *vide supra* or TEA•3HF for 24 h (Gasparutto *et al. Nucleic Acids Res.* 1992, 20, 5159-5166). Other alkylamine•HF complexes may also be used, e.g. trimethylamine or diisopropylethylamine.

5. The use of anion-exchange resins to purify and/or analyze the
10 fully deprotected RNA. These resins include, but are not limited to, quaternary or tertiary amino derivatized stationary phases such as silica or polystyrene. Specific examples include Dionex-NA100®, Mono-Q®, Poros-Q®.

- Thus, the invention features an improved method for the coupling of
15 RNA phosphoramidites; for the removal of amide or substituted amide protecting groups; and for the removal of 2'-hydroxyl alkylsilyl protecting groups. Such methods enhance the production of RNA or analogs of the type described above (e.g., with substituted 2'-groups), and allow efficient synthesis of large amounts of such RNA. Such RNA may also have
20 enzymatic activity and be purified without loss of that activity. While specific examples are given herein, those in the art will recognize that equivalent chemical reactions can be performed with the alternative chemicals noted above, which can be optimized and selected by routine experimentation.

- In another aspect, the invention features an improved method for the
25 purification or analysis of RNA or enzymatic RNA molecules (e.g. 28-70 nucleotides in length) by passing said RNA or enzymatic RNA molecule over an HPLC, e.g., reverse phase and/or an anion exchange chromatography column. The method of purification improves the catalytic activity of enzymatic RNAs over the gel purification method (see Figure 10).

- 30 Draper *et al.*, PCT WO93/23569, incorporated by reference herein, disclosed reverse phase HPLC purification. The purification of long RNA molecules may be accomplished using anion exchange chromatography, particularly in conjunction with alkali perchlorate salts. This system may be used to purify very long RNA molecules. In particular, it is advantageous to

use a Dionex NucleoPak 100[®] or a Pharmacia Mono Q[®] anion exchange column for the purification of RNA by the anion exchange method. This anion exchange purification may be used following a reverse-phase purification or prior to reverse phase purification. This method results in the
5 formation of a sodium salt of the ribozyme during the chromatography. Replacement of the sodium alkali earth salt by other metal salts, *e.g.*, lithium, magnesium or calcium perchlorate, yields the corresponding salt of the RNA molecule during the purification.

In the case of the 2-step purification procedure, in which the first step
10 is a reverse phase purification followed by an anion exchange step, the reverse phase purification is best accomplished using polymeric, *e.g.* polystyrene based, reverse-phase media, using either a 5'-trityl-on or 5'-trityl-off method. Either molecule may be recovered using this reverse-phase method, and then, once detritylated, the two fractions may be pooled
15 and then submitted to an anion exchange purification step as described above.

The method includes passing the enzymatically active RNA molecule over a reverse phase HPLC column; the enzymatically active RNA molecule is produced in a synthetic chemical method and not by an
20 enzymatic process; and the enzymatic RNA molecule is partially blocked, and the partially blocked enzymatically active RNA molecule is passed over a reverse phase HPLC column to separate it from other RNA molecules.

In more preferred embodiments, the enzymatically active RNA
25 molecule, after passage over the reverse phase HPLC column, is deprotected and passed over a second reverse phase HPLC column (which may be the same as the reverse phase HPLC column), to remove the enzymatic RNA molecule from other components. In addition, the column is a silica or organic polymer-based C4, C8 or C18 column having
30 a porosity of at least 125 Å, preferably 300 Å, and a particle size of at least 2 µm, preferably 5 µm.

Activation

The synthesis of RNA molecules may be accomplished chemically or enzymatically. In the case of chemical synthesis the use of tetrazole as an
35 activator of RNA phosphoramidites is known (Usman *et al.* *J. Am. Chem.*

Soc. 1987, 109, 7845-7854). In this, and subsequent reports, a 0.5 M solution of tetrazole is allowed to react with the RNA phosphoramidite and couple with the polymer bound 5'-hydroxyl group for 10 m. Applicant has determined that using 0.25-0.5 M solutions of 5-S-alkyltetrazoles for only 5 min gives equivalent or better results. The following exemplifies the procedure.

Example 7: Synthesis of RNA and Ribozymes Using 5-S-Alkyltetrazoles as Activating Agent

The method of synthesis used follows the general procedure for RNA synthesis as described in Usman et al., 1987*supra* and in Scaringe et al., *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The major difference used was the activating agent, 5-S-ethyl or -methyltetrazole @ 0.25 M concentration for 5 min.

All small scale syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for alkylsilyl protected RNA and 2.5 m coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite and a 40-fold excess of S-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

All large scale syntheses were conducted on a modified (eight amidite port capacity) 390Z (ABI) synthesizer using a 25 μ mol scale protocol with a 5-15 min coupling step for alkylsilyl protected RNA and 7.5 m coupling step for 2'-O-methylated RNA. A six-fold excess (1.5 mL of 0.1 M = 150 μ mol) of phosphoramidite and a forty-five-fold excess of S-ethyl tetrazole (4.5 mL of

0.25 M = 1125 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 390Z, determined by colorimetric quantitation of the trityl fractions, was 95.0-96.7%. Oligonucleotide synthesis reagents for the 390Z: Detritylation solution was 2% DCA in methylene chloride; capping was performed with 16% *N*-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25-0.5 M in acetonitrile) was made up from the solid obtained from Applied Biosystems.

Deprotection

The first step of the deprotection of RNA molecules may be accomplished by removal of the exocyclic amino protecting groups with either NH₄OH/EtOH:3/1 (Usman *et al.* *J. Am. Chem. Soc.* 1987, 109, 7845-7854) or NH₃/EtOH (Scarange *et al.* *Nucleic Acids Res.* 1990, 18, 5433-5341) for ~20 h @ 55-65 °C. Applicant has determined that the use of methylamine or NH₄OH/methylamine for 10-15 min @ 55-65 °C gives equivalent or better results. The following exemplifies the procedure.

Example 8: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using Methylamine (MA) or NH₄OH/Methylamine (AMA)

The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of methylamine (MA) or NH₄OH/methylamine (AMA) @ 55-65 °C for 5-15 min to remove the exocyclic amino protecting groups. The polymer-bound oligoribonucleotide was transferred from the synthesis column to a 4 mL glass screw top vial. NH₄OH and aqueous methylamine were pre-mixed in equal volumes. 4 mL of the resulting reagent was added to the vial, equilibrated for 5 m at RT and then heated at 55 or 65 °C for 5-15 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed with 1.0 mL of EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder. The same procedure was followed for the aqueous methylamine reagent.

Table 40 is a summary of the results obtained using the improvements outlined in this application for base deprotection.

- The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al. J. Am. Chem. Soc.* 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results. The following exemplifies this procedure.

Example 9: RNA and Ribozyme Deprotection of 2'-Hydroxyl Alkylsilyl Protecting Groups Using Anhydrous TEA•HF

- To remove the alkylsilyl protecting groups, the ammonia-deprotected oligoribonucleotide was resuspended in 250 µL of 1.4 M anhydrous HF solution (1.5 mL *N*-methylpyrrolidine, 750 µL TEA and 1.0 mL TEA•3HF) and heated to 65 °C for 1.5 h. 9 mL of 50 mM TEAB was added to quench the reaction. The resulting solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) prewashed with 10 mL of 50 mM TEAB. After washing the cartridge with 10 mL of 50 mM TEAB, the RNA was eluted with 10 mL of 2 M TEAB and dried down to a white powder.

Table 41 is a summary of the results obtained using the improvements outlined in this application for alkylsilyl deprotection.

Example 10: HPLC Purification, Anion Exchange column

- For a small scale synthesis, the crude material was diluted to 5 mL with diethylpyrocarbonate treated water. The sample was injected onto either a Pharmacia Mono Q® 16/10 or Dionex NucleoPac® column with 100% buffer A (10 mM NaClO₄). A gradient from 180-210 mM NaClO₄ at a rate of 0.85 mM/void volume for a Pharmacia Mono Q® anion-exchange column or 100-150 mM NaClO₄ at a rate of 1.7 mM/void volume for a Dionex NucleoPac® anion-exchange column was used to elute the RNA. Fractions were analyzed by a HP-1090 HPLC with a Dionex NucleoPac® column. Fractions containing full length product at ≥80% by peak area were pooled.
- For a trityl-off large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose® Fast Flow column. The column was thoroughly washed with 10 mM sodium perchlorate buffer. The oligonucleotide was eluted from the column with

300 mM sodium perchlorate. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material in the synthesis. The eluent was diluted four fold in sterile H₂O to lower the salt concentration and applied to a Pharmacia Mono Q[®] 16/10 column. A
5 gradient from 10-185 mM sodium perchlorate was run over 4 column volumes to elute shorter sequences, the full length product was then eluted in a gradient from 185-214 mM sodium perchlorate in 30 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac[®] column. Fractions containing over 85% full length material
10 were pooled. The pool was applied to a Pharmacia RPC[®] column for desalting.

For a trityl-on large scale synthesis, the crude material was desalted by applying the solution that resulted from quenching of the desilylation reaction to a 53 mL Pharmacia HiLoad 26/10 Q-Sepharose[®] Fast Flow
15 column. The column was thoroughly washed with 20 mM NH₄CO₃H/10% CH₃CN buffer. The oligonucleotide was eluted from the column with 1.5 M NH₄CO₃H/10% acetonitrile. The eluent was quantitated and an analytical HPLC was run to determine the percent full length material present in the synthesis. The oligonucleotide was then applied to a Pharmacia Resource
20 RPC column. A gradient from 20-55% B (20 mM NH₄CO₃H/25% CH₃CN, buffer A = 20 mM NH₄CO₃H/10% CH₃CN) was run over 35 column volumes. The fractions of interest were analyzed on a HP-1090 HPLC with a Dionex NucleoPac[®] column. Fractions containing over 60% full length material were pooled. The pooled fractions were then submitted to manual
25 detritylation with 80% acetic acid, dried down immediately, resuspended in sterile H₂O, dried down and resuspended in H₂O again. This material was analyzed on a HP 1090-HPLC with a Dionex NucleoPac[®] column. The material was purified by anion exchange chromatography as in the trityl-off scheme (*vide supra*).

30 Example 11 Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 μM, 200 nM, 40 nM or 8 nM and the final substrate RNA
35 concentrations were ~ 1 nM. Total reaction volumes were 50 μL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were

initiated by mixing substrate and ribozyme solutions at $t = 0$. Aliquots of 5 μL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each aliquot was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 12: One pot deprotection of RNA

Applicant has shown that aqueous methyl amine is an efficient reagent to deprotect bases in an RNA molecule. However, in a time consuming step (2-24 hrs), the RNA sample needs to be dried completely prior to the deprotection of the sugar 2'-hydroxyl groups. Additionally, deprotection of RNA synthesized on a large scale (e.g., 100 μmol) becomes challenging since the volume of solid support used is quite large. In an attempt to minimize the time required for deprotection and to simplify the process of deprotection of RNA synthesized on a large scale, applicant describes a one pot deprotection protocol (Fig. 12). According to this protocol, anhydrous methylamine is used in place of aqueous methyl amine. Base deprotection is carried out at 65 °C for 15 min and the reaction is allowed to cool for 10 min. Deprotection of 2'-hydroxyl groups is then carried out in the same container for 90 min in a TEA•3HF reagent. The reaction is quenched with 16 mM TEAB solution.

Referring to Fig. 13, hammerhead ribozyme targeted to site B is synthesized using RNA phosphoramidite chemistry and deprotected using either a two pot or a one pot protocol. Profiles of these ribozymes on an HPLC column are compared. The figure shows that RNAs deprotected by either the one pot or the two pot protocols yield similar full-length product profiles. Applicant has shown that using a one pot deprotection protocol, time required for RNA deprotection can be reduced considerably without compromising the quality or the yield of full length RNA.

Referring to Fig. 14, hammerhead ribozymes targeted to site B (from Fig. 13) are tested for their ability to cleave RNA. As shown in the figure 14, ribozymes that are deprotected using one pot protocol have catalytic activity comparable to ribozymes that are deprotected using a two pot protocol.

Example 12a: Improved protocol for the synthesis of phosphorothioate containing RNA and ribozymes using 5-S-Alkyltetrazoles as Activating Agent

5 The two sulfurizing reagents that have been used to synthesize ribophosphorothioates are tetraethylthiuram disulfide (TETD; Vu and Hirschbein, 1991 *Tetrahedron Letter* 31, 3005), and 3H-1,2-benzodithiol-3-one 1,1-dioxide (Beaucage reagent; Vu and Hirschbein, 1991 *supra*). TETD requires long sulfurization times (600 seconds for DNA and 3600 seconds for RNA). It has recently been shown that for sulfurization of DNA
10 oligonucleotides, Beaucage reagent is more efficient than TETD (Wyrzykiewicz and Ravikumar, 1994 *Bioorganic Med. Chem.* 4, 1519). Beaucage reagent has also been used to synthesize phosphorothioate oligonucleotides containing 2'-deoxy-2'-fluoro modifications wherein the wait time is 10 min (Kawasaki et al., 1992 *J. Med. Chem.*).

15 The method of synthesis used follows the procedure for RNA synthesis as described herein and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The sulfurization step for RNA described in the literature is a 8 second delivery and 10 min wait steps (Beaucage
20 and Iyer, 1991 *Tetrahedron* 49, 6123). These conditions produced about 95% sulfurization as measured by HPLC analysis (Morvan et al., 1990 *Tetrahedron Letter* 31, 7149). This 5% contaminating oxidation could arise from the presence of oxygen dissolved in solvents and/or slow release of traces of iodine adsorbed on the inner surface of delivery lines during
25 previous synthesis.

A major improvement is the use of an activating agent, 5-S-ethyltetrazole or 5-S-methyltetrazole at a concentration of 0.25 M for 5 min. Additionally, for those linkages which are phosphorothioate, the iodine
30 solution is replaced with a 0.05 M solution of 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent) in acetonitrile. The delivery time for the sulfurization step is reduced to 5 seconds and the wait time is reduced to 300 seconds.

RNA synthesis is conducted on a 394 (ABI) synthesizer using a modified 2.5 μ mol scale protocol with a reduced 5 min coupling step for
35 alkylsilyl protected RNA and 2.5 min coupling step for 2'-O-methylated RNA. A 6.5-fold excess (162.5 μ L of 0.1 M = 32.5 μ mol) of phosphoramidite

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and a 40-fold excess of *S*-ethyl tetrazole (400 μ L of 0.25 M = 100 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 synthesizer, determined by colorimetric quantitation of the trityl fractions, was 97.5-99%. Other oligonucleotide synthesis reagents for the 394 synthesizer: detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% *N*-Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle. *S*-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from Applied Biosystems. Sulfurizing reagent was obtained from Glen Research.

Average sulfurization efficiency (ASE) is determined using the formula: $ASE = (PS/Total)^{1/n-1}$

where, PS = integrated ³¹P NMR values of the P=S diester

Total = integration value of all peaks

n = length of oligo

Referring to tables 42 and 43, effects of varying the delivery and the wait time for sulfurization with Beaucage's reagent is described. These data suggest that 5 second wait time and 300 second delivery time is the condition under which ASE is maximum.

Using the above conditions a 36 mer hammerhead ribozyme is synthesized which is targeted to site C. The ribozyme is synthesized to contain phosphorothioate linkages at four positions towards the 5' end. RNA cleavage activity of this ribozyme is shown in Fig. 16. Activity of the phosphorothioate ribozyme is comparable to the activity of a ribozyme lacking any phosphorothioate linkages.

Example 13: Protocol for the synthesis of 2'-N-phthalimido-nucleoside phosphoramidite

The 2'-amino group of a 2'-deoxy-2'-amino nucleoside is normally protected with *N*-(9-fluorenylmethoxycarbonyl) (Fmoc; Imazawa and Eckstein, 1979 *supra*; Pleken et al., 1991 *Science* 253, 314). This protecting group is not stable in CH₃CN solution or even in dry form during

prolonged storage at -20 °C. These problems need to be overcome in order to achieve large scale synthesis of RNA.

Applicant describes the use of alternative protecting groups for the 2'-amino group of 2'-deoxy-2'-amino nucleoside. Referring to Figure 17,
5 phosphoramidite 17 was synthesized starting from 2'-deoxy-2'-aminonucleoside (12) using transient protection with Markievich reagent (Markiewicz *J. Chem. Res.* 1979, S, 24). An intermediate 13 was obtained in 50% yield, however subsequent introduction of N-phthaloyl (Pht) group by
Nefken's method (Nefkens, 1960 *Nature* 185, 306), desilylation (15),
10 dimethoxytrytilation (16) and phosphorylation led to phosphoramidite 17. Since overall yield of this multi-step procedure was low (20%) applicant investigated some alternative approaches, concentrating on selective introduction of N-phthaloyl group without acylation of 5' and 3' hydroxyls.

When 2'-deoxy-2'-amino-nucleoside was reacted with 1.05
15 equivalents of Nefkens reagent in DMF overnight with subsequent treatment with Et₃N (1 hour) only 10-15% of N and 5'(3')-bis-phthaloyl derivatives were formed with the major component being N-Pht-derivative 15. The N,O-bis by-products could be selectively and quantitatively converted to N-Pht derivative 15 by treatment of crude reaction mixture
20 with cat. KCN/MeOH.

A convenient "one-pot" procedure for the synthesis of key intermediate 16 involves selective N-phthaloylation with subsequent dimethoxytrytilation by DMTCl/Et₃N and resulting in the preparation of DMT derivative 16 in 85% overall yield as follows. Standard phosphorylation of
25 16 produced phosphoramidite 17 in 87% yield. One gram of 2'-amino nucleoside, for example 2'-amino uridine (US Biochemicals® part # 77140) was co-evaporated twice from dry dimethyl formamide (DMF) and dried in vacuo overnight. 50 mls of Aldrich sure-seal DMF was added to the dry 2'-amino uridine via syringe and the mixture was stirred for 10 minutes
30 to produce a clear solution. 1.0 grams (1.05 eq.) of N-carbethoxyphthalimide (Nefken's reagent, 98% Janssen Chimica) was added and the solution was stirred overnight. Thin layer chromatography (TLC) showed 90% conversion to a faster moving products (10% ETOH in CHCl₃) and 57 µl of TEA (0.1 eq.) was added to effect closure of the phthalimide ring. After 1 hour an additional 855 µl (1.5 eq.) of TEA was
35 added followed by the addition of 1.53 grams (1.1 eq.) of DMT-Cl

(Lancaster Synthesis®, 98%). The reaction mixture was left to stir overnight and quenched with ETOH after TLC showed greater than 90% desired product. Dmf was removed under vacuum and the mixture was washed with sodium bicarbonate solution (5% aq., 500 mls) and extracted with ethyl acetate (2x 200 mls). A 25mm x 300mm flash column (75 grams Merck flash silica) was used for purification. Compound eluted at 80 to 85% ethyl acetate in hexanes (yield: 80% purity: >95% by ¹HNMR). Phosphoramidites were then prepared using standard protocols described above.

- 10 With phosphoramidite 17 in hand applicant synthesized several ribozymes with 2'-deoxy-2'-amino modifications. Analysis of the synthesis demonstrated coupling efficiency in 97-98% range. RNA cleavage activity of ribozymes containing 2'-deoxy-2'-amino-U modifications at U4 and/or U7 positions (see Figure 1), wherein the 2'-amino positions were either
15 protected with Fmoc or Pht, was identical. Additionally, complete deprotection of 2'-deoxy-2'-amino-Uridine was confirmed by base-composition analysis. The coupling efficiency of phosphoramidite 17 was not effected over prolonged storage (1-2 months) at low temperatures.

Protecting 2' Position with a SEM Group

- 20 There follows a method using the 2'-(trimethylsilyl)ethoxymethyl protecting group (SEM) in the synthesis of oligoribonucleotides, and in particular those enzymatic molecules described above. For the synthesis of RNA it is important that the 2'-hydroxyl protecting group be stable throughout the various steps of the synthesis and base deprotection. At the
25 same time, this group should also be readily removed when desired. To that end the *t*-butyldimethylsilyl group has been efficacious (Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990, 18, 5433-5441). However, long exposure times to tetra-*n*-butylammonium fluoride (TBAF) are generally required to fully remove this
30 protecting group from the 2'-hydroxyl. In addition, the bulky alkyl substituents can prove to be a hindrance to coupling thereby necessitating longer coupling times. Finally, it has been shown that the TBDMS group is base labile and is partially deprotected during treatment with ethanolic ammonia (Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.* 1990,
35

18, 5433-5441 and Stawinski,J.; Stromberg,R.; Thelin,M.; Westman,E. *Nucleic Acids Res.* 1988, 16, 9285-9298).

5 The (trimethylsilyl)ethoxymethyl ether (SEM) seems a suitable substitute. This protecting group is stable to base and all but the harshest acidic conditions. Therefore it is stable under the conditions required for oligonucleotide synthesis. It can be readily introduced and the oxygen carbon bond makes it unable to migrate. Finally, the SEM group can be removed with $\text{BF}_3 \cdot \text{OEt}_2$ very quickly.

10 There follows a method for synthesis of RNA by protecting the 2'-position of a nucleotide during RNA synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group. The method can involve use of standard RNA synthesis conditions as discussed below, or any other equivalent steps. Those in the art are familiar with such steps. The nucleotide used can be any normal nucleotide or may be substituted in
15 various positions by methods well known in the art, e.g., as described by Eckstein *et al.*, *International Publication* No. WO 92/07065, Perrault *et al.*, *Nature* 1990, 344, 565-568, Pieken *et al.*, *Science* 1991, 253, 314-317, Usman,N.; Cedergren,R.J. *Trends in Biochem. Sci.* 1992, 17, 334-339, Usman *et al.*, PCT WO93/15187, and Sproat,B. *European Patent*
20 *Application* 92110298.4 .

This invention also features a method for covalently linking a SEM group to the 2'-position of a nucleotide. The method involves contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions. In a preferred embodiment, the conditions are dibutyltin oxide,
25 tetrabutylammonium fluoride and SEM-Cl. Those in the art, however, will recognize that other equivalent conditions can also be used.

In another aspect, the invention features a method for removal of an SEM group from a nucleoside molecule or an oligonucleotide. The method involves contacting the molecule or oligonucleotide with boron trifluoride
30 etherate ($\text{BF}_3 \cdot \text{OEt}_2$) under SEM removing conditions, e.g., in acetonitrile.

Referring to Figure 18, there is shown the method for solid phase synthesis of RNA. A 2',5'-protected nucleotide is contacted with a solid phase bound nucleotide under RNA synthesis conditions to form a dinucleotide. The protecting group (R) at the 2'-position in prior art

methods can be a silyl ether, as shown in the Figure. In the method of the present invention, an SEM group is used in place of the silyl ether. Otherwise RNA synthesis can be performed by standard methodology.

Referring to Figure 19, there is shown the synthesis of 2'-O-SEM protected nucleosides and phosphoramidites. Briefly, a 5'-protected nucleoside (1) is protected at the 2'- or 3'-position by contacting with a derivative of SEM under appropriate conditions. Specifically, those conditions include contacting the nucleoside with dibutyltin oxide and SEM chloride. The 2 regioisomers are separated by chromatography and the 2'-protected moiety is converted into a phosphoramidite by standard procedure. The 3'-protected nucleoside is converted into a succinate derivative suitable for derivatization of a solid support.

Referring to Figure 20, a prior art method for deprotection of RNA using silyl ethers is shown. This contrasts with the method shown in Figure 21 in which deprotection of RNA containing an SEM group is performed. In step 1, the base protecting groups and cyanoethyl groups are removed by standard procedure. The SEM group is then removed as shown in the Figure. The details of the synthesis of phosphoramidites and SEM protected nucleosides and their use in synthesis of oligonucleotides and subsequent deprotection of

Example 14: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Dimethoxytrityl Uridine (2)

Referring to Figure 19, 5'-O-dimethoxytrityl uridine 1 (1.0 g, 1.83 mmol) in CH₃CN (18 mL) was added dibutyltin oxide (1.0 g, 4.03 mmol) and TBAF (1 M, 2.38 mL, 2.38 mmol). The mixture was stirred for 2 h at RT (about 20-25°C) at which time (trimethylsilyl)ethoxymethyl chloride (SEM-Cl) (487 µL, 2.75 mmol) was added. The reaction mixture was stirred overnight and then filtered and evaporated. Flash chromatography (30% hexanes in ethyl acetate) yielded 347 mg (28.0%) of 2'-hydroxyl protected nucleoside 2 and 314 mg (25.3%) of 3'-hydroxyl protected nucleoside 3.

Example 15: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl) Uridine (4)

Nucleoside 2 was detritylated following standard methods, as shown in Figure 19.

Example 16: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5',3'-O-Acetyl Uridine (5)

Nucleoside 4 was acetylated following standard methods, as shown in Figure 19.

5 Example 17: Synthesis of 5',3'-O-Acetyl Uridine (6)

Referring to Figure 19, the fully protected uridine 5 (32 mg, 0.07 mmol) was dissolved in CH₃CN (700 µL) and BF₃•OEt₂ (17.5 µL, 0.14 mmol) was added. The reaction was stirred 15 m and MeOH was added to quench the reaction. Flash chromatography (5% MeOH in CH₂Cl₂) gave
10 20 mg (88%) of SEM deprotected nucleoside 6.

Example 18: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-3'-O-Succinyl-5'-O-Dimethoxytrityl Uridine (2)

Nucleoside 3 was succinylated and coupled to the support following standard procedures, as shown in Figure 19.

15 Example 19: Synthesis of 2'-O-((trimethylsilyl)ethoxymethyl)-5'-O-Dimethoxytrityl Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (8)

Nucleoside 3 was phosphitylated following standard methods, as shown in Figure 19.

20 Example 20: Synthesis of RNA Using 2'-O-SEM Protection

Referring to Figure 18, the method of synthesis used follows the general procedure for RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucl. Acids Res.*
25 1990, 18, 5433-5441. The phosphoramidite 8 was coupled following standard RNA methods to provide a 10-mer of uridylic acid. Syntheses were conducted on a 394 (ABI) synthesizer using a modified 2.5 µmol scale protocol with a 10 m coupling step. A thirteen-fold excess (325 µL of 0.1 M = 32.5 µmol) of phosphoramidite and a 80-fold excess of tetrazole
30 (400 µL of 0.5 M = 200 µmol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394, determined by colorimetric quantitation of the trityl fractions, were 98-99%. Other oligonucleotide synthesis reagents for the 394: Detritylation solution was 2% TCA in methylene chloride; capping was performed with 16% N-

Methyl imidazole in THF and 10% acetic anhydride/10% 2,6-lutidine in THF; oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF. Fisher Synthesis Grade acetonitrile was used directly from the reagent bottle.

- 5 Referring to Figure 21, the homopolymer was base deprotected with NH₃/EtOH at 65 °C. The solution was decanted and the support was washed twice with a solution of 1:1:1 H₂O:CH₃CN:MeOH. The combined solutions were dried down and then diluted with CH₃CN (1 mL). BF₃•OEt₂ (2.5 µL, 30 µmol) was added to the solution and aliquots were removed at
10 ten time points. The results indicate that after 30 min deprotection is complete, as shown in Figure 22.

III. Vectors Expressing Ribozymes

- There follows a method for expression of a ribozyme in a bacterial or eucaryotic cell, and for production of large amounts of such a ribozyme. In
15 general, the invention features a method for preparing multi-copy cassettes encoding a defined ribozyme structure for production of a ribozyme at a decreased cost. A vector is produced which encodes a plurality of ribozymes which are cleaved at their 3' and 5' ends from an RNA transcript produced from the vector by only one other ribozyme. The system is useful
20 for scaling up production of a ribozyme, which may be either modified or unmodified, *in situ* or *in vitro*. Such vector systems can be used to express a desired ribozyme in a specific cell, or can be used in an *in vitro* system to allow production of large amounts of a desired ribozyme. The vectors of this invention allow a higher yield synthesis of a ribozyme in the form of an
25 RNA transcript which is cleaved *in situ* or *in vitro* before or after transcript isolation.

- Thus, this invention is distinct from the prior art in that a single ribozyme is used to process the 3' and 5' ends of each therapeutic, trans-
acting or desired ribozyme instead of processing only one end, or only one
30 ribozyme. This allows smaller vectors to be derived with multiple trans-acting ribozymes released by only one other ribozyme from the mRNA transcript. Applicant has also provided methods by which the activity of such ribozymes is increased compared to those in the art, by designing ribozyme-encoding vectors and the corresponding transcript such that

folding of the mRNA does not interfere with processing by the releasing ribozyme.

The stability of the ribozyme produced in this method can be enhanced by provision of sequences at the termini of the ribozymes as described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein.

The method of this invention is advantageous since it provides high yield synthesis of ribozymes by use of low cost transcription-based protocols, compared to existing chemical ribozyme synthesis, and can use isolation techniques currently used to purify chemically synthesized oligonucleotides. Thus, the method allows synthesis of ribozymes in high yield at low cost for analytical, diagnostic, or therapeutic applications.

The method is also useful for synthesis of ribozymes *in vitro* for ribozyme structural studies, enzymatic studies, target RNA accessibility studies, transcription inhibition studies and nuclease protection studies, much is described by Draper et al., PCT WO 93/23509 hereby incorporated by reference herein.

The method can also be used to produce ribozymes *in situ* either to increase the intracellular concentration of a desired therapeutic ribozyme, or to produce a concatameric transcript for subsequent *in vitro* isolation of unit length ribozyme. The desired ribozyme can be used to inhibit gene expression in molecular genetic analyses or in infectious cell systems, and to test the efficacy of a therapeutic molecule or treat afflicted cells.

Thus, in general, the invention features a vector which includes a bacterial, viral or eucaryotic promoter within a plasmid, cosmid, phagmid, virus, viroid, virusoid or phage vector. Other vectors are equally suitable and include double-stranded, or partially double-stranded DNA, formed by an amplification method such as the polymerase chain reaction, or double-stranded, partially double-stranded or single-stranded RNA, formed by site-directed homologous recombination into viral or viroid RNA genomes. Such vectors need not be circular. Transcriptionally linked to the promoter region is a first ribozyme-encoding region, and nucleotide sequences encoding a ribozyme cleavage sequence which is placed on either side of a region encoding a therapeutic or otherwise desired second ribozyme.

Suitable restriction endonuclease sites can be provided to ease construction of this vector in DNA vectors or in requisite DNA vectors of an RNA expression system. The desired second ribozyme may be any desired type of ribozyme, such as a hammerhead, hairpin, hepatitis delta virus (HDV) or other catalytic center, and can include group I and group II introns, as discussed above. The first ribozyme is chosen to cleave the encoded cleavage sequence, and may also be any desired ribozyme, for example, a *Tetrahymena* derived ribozyme, which may, for example, include an imbedded restriction endonuclease site in the center of a self-recognition sequence to aid in vector construction. This endonuclease site is useful for construction of the vector, and subsequent analysis of the vector.

When the promoter of such a vector is activated an RNA transcript is produced which includes the first and second ribozyme sequences. The first ribozyme sequence is able to act, under appropriate conditions, to cause cleavage at the cleavage sites to release the second ribozyme sequences. These second ribozyme sequences can then act at their target RNA sites, or can be isolated for later use or analysis.

Thus, in one aspect the invention features a vector which includes a first nucleic acid sequence (encoding a first ribozyme having intramolecular cleaving activity), and a second nucleic acid sequence (encoding a second ribozyme having intermolecular cleaving enzymatic activity) flanked by nucleic acid sequences encoding RNA which is cleaved by the first ribozyme to release the second ribozyme from the RNA transcript encoded by the vector. The second ribozyme may be flanked by the first ribozyme either on the 5' side or 3' side. If desired, the first ribozyme may be encoded on a separate vector and may have intermolecular cleaving activity.

As discussed above, the first ribozyme can be chosen to be any self-cleaving ribozyme, and the second ribozyme may be chosen to be any desired ribozyme. The flanking sequences are chosen to include sequences recognized by the first ribozyme. When the vector is caused to express RNA from these nucleic acid sequences, that RNA has the ability under appropriate conditions to cleave each of the flanking regions and thereby release one or more copies of the second ribozyme. If desired, several different second ribozymes can be produced by the same vector, or

several different vectors can be placed in the same vessel or cell to produce different ribozymes.

In preferred embodiments, the vector includes a plurality of the nucleic acid sequences encoding the second ribozyme, each flanked by nucleic acid sequences recognized by the first ribozyme. Most preferably, such a plurality includes at least six to nine or even between 60 - 100 nucleic acid sequences. In other preferred embodiments, the vector includes a promoter which regulates expression of the nucleic acid encoding the ribozymes from the vector, and the vector is chosen from a plasmid, cosmid, phagmid, virus, viroid or phage. In a most preferred embodiment, the plurality of nucleic acid sequences are identical and are arranged in sequential order such that each has an identical end nearest to the promoter. If desired, a poly(A) sequence adjacent to the sequence encoding the first or second ribozyme may be provided to increase stability of the RNA produced by the vector, and a restriction endonuclease site adjacent to the nucleic acid encoding the first ribozyme is provided to allow insertion of nucleic acid encoding the second ribozyme during construction of the vector.

In a second aspect, the invention features a method for formation of a ribozyme expression vector by providing a vector including nucleic acid encoding a first ribozyme, as discussed above, and providing a single-stranded DNA encoding a second ribozyme, as discussed above. The single-stranded DNA is then allowed to anneal to form a partial duplex DNA which can be filled in by a treatment with an appropriate enzyme, such as a DNA polymerase in the presence of dNTPs, to form a duplex DNA which can then be ligated to the vector. Large vectors resulting from this method can then be selected to insure that a high copy number of the single-stranded DNA encoding the second ribozyme is incorporated into the vector.

In a further aspect, the invention features a method for production of ribozymes by providing a vector as described above, expressing RNA from that vector, and allowing cleavage by the first ribozyme to release the second ribozyme.

In preferred embodiments, three different ribozyme motifs are used as cis-cleaving ribozymes. The hammerhead, hairpin, and hepatitis delta

virus (HDV) ribozyme motifs consist of small, well-defined sequences that rapidly self-cleave *in vitro* (Symons, 1992 Annu. Rev. Biochem. 61, 641). While structural and functional differences exist among the three ribozyme motifs, they self-process efficiently *in vivo*. All three ribozyme motifs self-process to 87-95% completion in the absence of 3' flanking sequences. *In vitro*, the self-processing constructs described in this invention are significantly more active than those reported by Taira et al., 1990 supra; and Altschuler et al., 1992 Gene 122, 85. The present invention enables the use of cis-cleaving ribozymes to efficiently truncate RNA molecules at specific sites *in vivo* by ensuring lack of secondary structure which prevents processing.

Isolation of Therapeutic Ribozyme

The preferred method of isolating therapeutic ribozyme is by a chromatographic technique. The HPLC purification methods and reverse HPLC purification methods described by Draper et al., PCT WO 93/23509, hereby incorporated by reference herein, can be used. Alternatively, the attachment of complementary oligonucleotides to cellulose or other chromatography columns allows isolation of the therapeutic second ribozyme, for example, by hybridization to the region between the flanking arms and the enzymatic RNA. This hybridization will select against the short flanking sequences without the desired enzymatic RNA, and against the releasing first ribozyme. The hybridization can be accomplished in the presence of a chaotropic agent to prevent nuclease degradation. The oligonucleotides on the matrix can be modified to minimize nuclease activity, for example, by provision of 2'-O-methyl RNA oligonucleotides. Such modifications of the oligonucleotide attached to the column matrix will allow the multiple use of the column with minimal oligo degradation. Many such modifications are known in the art, but a chemically stable non-reducible modification is preferred. For example, phosphorothioate modifications can also be used.

The expressed ribozyme RNA can be isolated from bacterial or eucaryotic cells by routine procedures such as lysis followed by guanidine isothiocyanate isolation.

The current known self-cleaving site of *Tetrahymena* can be used in an alternative vector of this invention. If desired, the full-length

Tetrahymena sequence may be used, or a shorter sequence may be used. It is preferred that, in order to decrease the superfluous sequences in the self-cleaving site at the 5' cleavage end, the hairpin normally present in the *Tetrahymena* ribozyme should contain the therapeutic second ribozyme 3' sequence and its complement. That is, the first releasing ribozyme-encoding DNA is provided in two portions, separated by DNA encoding the desired second ribozyme. For example, if the therapeutic second ribozyme recognition sequence is CGGACGA/CGAGGA, then CGAGGA is provided in the self-cleaving site loop such that it is in a stem structure recognized by the *Tetrahymena* ribozyme. The loop of the stem may include a restriction endonuclease site into which the desired second ribozyme-encoding DNA is placed.

If desired, the vector may be used in a therapeutic protocol by use of the systems described by Lechner, PCT WO 92/13070, hereby incorporated by reference herein, to allow a timed expression of the therapeutic second ribozyme, as well as an appropriate shut off of cell or gene function. Thus, the vector will include a promoter which appropriately expresses enzymatically active RNA only in the presence of an RNA or another molecule which indicates the presence of an undesired organism or state. Such enzymatically active RNA will then kill or harm the cell in which it exists, as described by Lechner, *id.*, or act to cause reduced expression of a desired protein product.

A number of suitable RNA vectors may also be used in this invention. The vectors include plant viroids, plant viruses which contain single or double-stranded RNA genomes and animal viruses which contain RNA genomes, such as the picomaviruses, myxoviruses, paramyxoviruses, hepatitis A virus, reovirus and retroviruses. In many instances cited, use of these viral vectors also results in tissue specific delivery of the ribozymes.

Example 21: Design of self-processing cassettes

In a preferred embodiment, applicant compared the *in vitro* and *in vivo* cis-cleaving activity of three different ribozyme motifs—the hammerhead, the hairpin and the hepatitis delta virus ribozyme—in order to assess their potential to process the ends of transcripts *in vivo*. To make a direct comparison among the three, however, it is important to design the ribozyme-containing transcripts to be as similar as possible. To this end,

all the ribozyme cassettes contained the same trans-acting hammerhead ribozyme followed immediately by one of the three cis-acting ribozymes (Figure 23-25). For simplicity, applicant refers to each cassette by an abbreviation that indicates the downstream cis-cleaving ribozyme only.

- 5 Thus HH refers to the cis-cleaving cassette containing a hammerhead ribozyme, while HP and HDV refer to the cassettes containing hairpin and hepatitis delta virus cis-cleaving ribozymes, respectively. The general design of the ribozyme cassettes, as well as specific differences among the cassettes, are outlined below.

- 10 A sequence predicted to form a stable stem-loop structure is included at the 5' end of all the transcripts. The hairpin stem contains the T7 RNA polymerase initiation sequence (Milligan & Uhlenbeck, 1989 Methods Enzymol. 180, 51) and its complement, separated by a stable tetra-loop (Antao et al., 1991 Nucleic Acids Res. 19, 5901). By incorporating the T7
15 initiation sequence into a stem-loop structure, applicant hoped to avoid nonproductive base pairing interactions with either the trans-acting ribozyme or with the cis-acting ribozyme. The presence of a hairpin at the end of a transcript may also contribute to the stability of the transcript *in vivo*. These are non-limiting examples. Those in the art will recognize that
20 other embodiments can be readily generated using a variety of promoters, initiator sequences and stem-loop structure combinations generally known in the art.

- The trans-acting ribozyme used in this study is targeted to a site B (5'...CUGGAGUC↓GACCUUC...3'). The 5' binding arm of the ribozyme, 5'-
25 GAAGGUC-3', and the core of the ribozyme, 5'-CUGAUGAGGCCGAAAGGCCGAA-3', remain constant in all cases. In addition, all transcripts also contain a single nucleotide between the 5' stem-loop and the first nucleotide of the ribozyme. The linker nucleotide was required to obtain the same activity *in vitro* that was measured with an
30 identical ribozyme lacking the 5' hairpin. Because the three cis-cleaving ribozymes have different requirements at the site of cleavage, slight differences were unavoidable at the 3' end of the processed transcript. The junction between the trans- and cis-acting ribozyme is, however, designed so that there is minimal extraneous sequence left at the 3' end of the trans-
35 cleaving ribozyme once cis-cleavage occurs. The only differences between the constructs lie in the 3' binding arm of the ribozyme, where

either 6 or 7 nucleotides, 5'-ACUCCA(+/-G)-3', complementary to the target sequence are present and where, after processing, two to five extra nucleotides remain.

5 The cis-cleaving hammerhead ribozyme used in the HH cassette is based on the design of Grosshans and Cech, 1991 supra. As shown in Figure 23, the 3' binding arm of the trans-acting ribozyme is included in the required base-pairing interactions of the cis-cleaving ribozyme to form stem I. Two extra nucleotides, UC, were included at the end of the 3' binding arm to form the self-processing hammerhead ribozyme site (Ruffner et al.,
10 1990 supra) which remain on the 3' end of the trans-acting ribozyme following self-processing.

The hairpin ribozyme portion of the HP self-processing construct is based on the minimal wild-type sequence (Hampel & Tritz, 1989 supra). A tetra-loop at the end of helix 1 (3' side of the cleavage site) serves to link
15 the two portions and thus allows a minimal five nucleotides to remain at the end of the released trans-acting ribozyme following self-processing. Two variants of HP were designed: HP(GU) and HP(GC). The HP(GU) was constructed with a G-U wobble base pair in helix 2 (A52G substitution; Figure 24). This slight destabilization of helix 2 was intended to improve
20 self-processing activity by promoting product release and preventing the reverse reaction (Berzal-Herranz et al., 1992 Genes & Dev. 6, 129; Chowrira et al., 1993 Biochemistry 32, 1088). The HP(GC) cassette was constructed as a control for strong base-pairing interactions in helix 2 (U77C and A52G substitution; Figure 24). Another modification to
25 discourage the reverse ligation reaction of the hairpin ribozyme was to shorten helix 1 (Figure 24) by one base pair relative to the wild-type sequence (Chowrira & Burke, 1991 Biochemistry 30, 8518).

The HDV ribozyme self-processes efficiently when the nucleotide 5' to the cleavage site is a pyrimidine, and somewhat less so when adenosine is
30 in that position. No other sequence requirements have been identified upstream of the cleavage site, however, we have observed some decrease in activity when a stem-loop structure was present within 2 nt of the cleavage site. The HDV self-processing construct (Fig 25) was designed to generate the trans-acting hammerhead ribozyme with only two additional
35 nucleotides at its 3' end after self-processing. The HDV sequence used here is based on the anti-genomic sequence (Perrota & Been, 1992 supra)

but includes the modifications of Been et al., 1992 (Biochemistry 31, 11843) in which cis-cleavage activity of the ribozyme was improved by the substitution of a shortened helix 4 for a wild-type stem-loop (Figure 25).

5 To prepare DNA inserts that encode self-processing ribozyme cassettes, partially overlapping top- and bottom-strand oligonucleotides (60-90 nucleotides) were designed to include sequences for the T7 promoter, the trans-acting ribozyme, the cis-cleaving ribozyme and appropriate restriction sites for use in cloning (see Fig. 26). The single-strand portions of annealed oligonucleotides were converted to double-
10 strands using Sequenase® (U.S. Biochemicals). Insert DNA was ligated into *EcoRI*/*HindIII*-digested puc18 and transformed into *E. coli* strain DH5α using standard protocols (Maniatis et al., 1982 in Molecular Cloning Cold Spring Harbor Press). The identity of positive clones was confirmed by sequencing small-scale plasmid preparations.

15 Larger scale preparations of plasmid DNA for use as *in vitro* transcription templates and in transactions were prepared using the protocol and columns from QIAGEN Inc. (Studio City, CA) except that an additional ethanol precipitation was included as the final step.

Example 22: RNA Processing *in vitro*

20 Transcription reactions containing linear plasmid templates were carried out essentially as described (Milligan & Uhlenbeck, 1989 Supra; Chowrira & Burke, 1991 Supra). In order to prepare 5' end-labeled transcripts, standard transcription reactions were carried out in the presence of 10-20 μCi [γ -³²P]GTP, 200 μM each NTP and 0.5 to 1 μg of
25 linearized plasmid template. The concentration of MgCl₂ was maintained at 10 mM above the total nucleotide concentration.

To compare the ability of the different ribozyme cassettes to self-process *in vitro*, each construct was transcribed and allowed to undergo self-processing under identical conditions at 37°C. For these comparisons,
30 equal amounts of linearized DNA templates bearing the various ribozyme cassettes were transcribed in the presence of [γ -³²P]GTP to generate 5' end-labeled transcripts. In this manner only the full-length, unprocessed transcripts and the released trans-ribozymes are visualized by autoradiography. In all reactions, Mg²⁺ was included at 10 mM above the
35 nucleotide concentration so that cleavage by all the ribozyme cassettes

would be supported. Transcription templates were linearized at several positions by digestion with different restriction enzymes so that self-processing in the presence of increasing lengths of downstream sequence could be compared (see Fig. 26). The resulting transcripts have either 4-5 non-ribozyme nucleotides at the 3' end (*Hind*III-digested template), 220 nucleotides (*Nde*I digested templates) or 454 nucleotides of downstream sequence (*Rca*I digested template).

As shown in Figure 27, all four ribozyme cassettes are capable of self-processing and yield RNA products of expected sizes. Two nucleotides essential for hammerhead ribozyme activity (Ruffner et al., 1990 *supra*) have been changed in the HH(mutant) core sequence (see Figure 23) and so this transcript is unable to undergo self-processing (Fig. 27). This is evidenced by the lack of a released 5' RNA in the HH(mutant), although the full-length RNAs are present. Comparison of the amounts of released trans-ribozyme (Fig. 27) indicate that there are differences in the ability of these ribozymes to self-process *in vitro*, especially with respect to the presence of downstream sequence. For the two HP constructs, it is clear that HP(GC) is more efficient than the HP(GU) ribozyme, both in the presence and in the absence of extra downstream sequence. In addition, the activity of HP(GU) falls off more dramatically when downstream sequence is present. The stronger G:C base pair likely contributes to the HP(GC) construct's ability to fold correctly (and/or more quickly) into the productive structure, even when as much as 216 extra nucleotides are present downstream. The HH ribozyme construct is also quite efficient at self-processing, and slightly better than the HP(GU) construct even when downstream sequence is present.

Of the three ribozyme motifs, the presence of extra downstream sequence seems to most affect the efficiency of HDV. When no extra sequence is present downstream, HDV is quite efficient and self-processes to approximately the same level as the HH and HP(GC) cassettes. However, when extra downstream sequence is present, the self-processing activity seems to decrease almost as dramatically as is seen with the (sub-optimal) HP(GU) cassette.

Example 23: Kinetics of self-processing reaction

*Hind*III-digested template (250 ng) was used in a standard transcription reaction mixture containing: 50 mM Tris-HCl pH 8.3; 1 mM ATP, GTP and UTP; 50 μ M CTP; 40 μ Ci [α -³²P]CTP; 12 mM MgCl₂; 10 mM DTT. The transcription/self-processing reaction was initiated by the addition of T7 RNA polymerase (15 U/ μ l). Aliquots of 5 μ l were taken at regular time intervals and the reaction was stopped by adding an equal volume of 2x formamide loading buffer (95% formamide, 15 mM EDTA, & dyes) and freezing on dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel and results were quantitated by PhosphorImager (Molecular Dynamics, Sunnyvale, CA). Ribozyme self-cleavage rates were determined from non-linear, least-squares fits (KaleidaGraph, Synergy Software, Reading, PA) of the data to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

where *t* represents time and *k* represents the unimolecular rate constant for cleavage (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977).

Linear templates were prepared by digesting the plasmids with *Hind*III so that transcripts will contain only four to five vector-derived nucleotides at the 3' end (see Figure 23-25). By comparison of the unimolecular rate constant (*k*) determined for each construct, it is clear that HH is the most efficient at self-processing (Table 44). The HH transcript self-processes 2-fold faster than HDV and 3-fold faster than HP(GC) transcripts. Although the HP(GU) RNA undergoes self-processing, it is at least 6-fold slower than the HP(GC) construct. This is consistent with previous observations that the stability of helix 2 is essential for self-processing and trans-cleavage activity of the hairpin ribozyme (Hampel et al., 1990 supra; Chowrira & Burke, 1991 supra). The rate of HH self-cleavage during transcription measured here (1.2 min⁻¹) is similar to the rate measured by Long and Uhlenbeck 1994 supra using a HH that has a different stem I and stem III. Self-processing rates during transcription for HP and HDV have not been previously reported. However, self-processing of the HDV ribozyme—as measured here during transcription—is significantly slower than when tested after isolation from a denaturing gel (Been et al., 1992 supra). This decrease likely reflects the difference in protocol as well as the presence of 5' flanking sequence in the HDV construct used here.

Example 24: Effect of downstream sequences on trans-cleavage *in vitro*

Transcripts containing the trans ribozyme with or without 3' flanking sequences were assayed for their ability to cleave their target in trans. To this end, transcripts from three templates were resolved on a preparative gel and bands corresponding both to processed trans-acting ribozymes from the HH transcription reaction, and to full-length HH(mutant) and Δ HDV transcripts were isolated. In all three transcripts the trans-acting ribozyme portion is identical—with the exception of sequences at their 3' ends. The HH trans-acting ribozyme contains only an additional UC at its 3' end, while HH(mutant) and Δ HDV have 52 and 37 nucleotides, respectively, at their 3' ends. A 622 nucleotide, internally-labeled target RNA was incubated, under ribozyme excess conditions, along with the three ribozyme transcripts in a standard reaction buffer.

To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 622 nt region (containing hammerhead site P) was synthesized by PCR using primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed in a standard transcription buffer in the presence of [α - 32 P]CTP (Chowrira & Burke, 1991 *supra*). The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (1 μ M) and internally labeled 622 nt substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager[®] (Molecular Dynamics, Sunnyvale, CA).

The HH trans-acting ribozyme cleaves the target RNA approximately 10-fold faster than the Δ HDV transcript and greater than 20-fold faster than

the HH(mutant) transcript (Figure 28). The additional nucleotides at the end of HH(mutant) form 7 base-pairs with the 3' target-binding arm of the trans-acting ribozyme (Figure 23). This interaction must be disrupted (at a cost of 6 kcal/mole) to make the trans-acting ribozyme available for binding the target sequence. In contrast, the additional nucleotides at the end of Δ HDV were not designed to form any strong, alternative base-pairing with the trans-ribozyme. Nevertheless, the Δ HDV sequences are predicted to form multiple structures involving the 3' target-binding arm of the trans ribozyme that have stabilities ranging from 1-2 kcal/mole. Thus, the observed reductions in activity for the Δ HDV and HH(mutant) constructs are consistent with the predicted folded structures, and it reinforces the view that the flanking sequences can decrease the catalytic efficiency of a ribozyme through nonproductive interactions with either the ribozyme or the substrate or both.

Example 25: RNA self-processing *in vivo*

Since three of the constructs (HH, HDV and HP(GC)) self-process efficiently in solution, the affect of the mammalian cellular milieu on ribozyme self-processing was next explored by applicant. A transient expression system was employed to investigate ribozyme activity *in vivo*. A mouse cell line (OST7-1) that constitutively expresses T7 RNA polymerase in the cytoplasm was chosen for this study (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. USA 87, 6743). In these cells plasmids containing a ribozyme cassette downstream of the T7 promoter will be transcribed efficiently in the cytoplasm (Elroy-Stein & Moss, 1990 supra).

Monolayers of a mouse L9 fibroblast cell line (OST7-1; Elroy-Stein and Moss, 1990 supra) were grown in 6-well plates with $\sim 5 \times 10^5$ cells/well. Cells were transfected with circular plasmids (5 μ g/well) using the calcium phosphate-DNA precipitation method (Maniatis et al., 1982 supra). Cells were lysed (4 hours post-transfection) by the addition of standard lysis buffer (200 μ l/well) containing 4M guanadinium isothiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% sarkosyl (Chomczynski and Sacchi, 1987 Anal. Biochem. 162, 156), and 50 mM EDTA pH 8.0. The lysate was extracted once with water-saturated phenol followed by one extraction with chloroform:isoamyl alcohol (25:1). Total cellular RNA was precipitated with an equal volume of isopropanol. The RNA pellet was resuspended in 0.2

M ammonium acetate and reprecipitated with ethanol. The pellet was then washed with 70% ethanol and resuspended in DEPC-treated water.

Purified cellular RNA (3 µg/reaction) was first denatured in the presence of a 5' end-labeled DNA primer (100 pmol) by heating to 90°C for 2 min. in the absence of Mg²⁺, and then snap-cooling on ice for at least 15 min. This protocol allows for efficient annealing of the primer to its complementary RNA sequence. The primer was extended using Superscript II reverse transcriptase (8 U/µl; BRL) in a buffer containing 50 mM Tris-HCl pH 8.3; 10 mM DTT; 75 mM KCl; 1 mM MgCl₂; 1 mM each dNTP. The extension reaction was carried out at 42°C for 10 min. The reaction was terminated by adding an equal volume of 2x formamide gel loading buffer and freezing on crushed dry ice. The samples were resolved on a 10% polyacrylamide sequencing gel. The primer sequences are as follows: HH primer, 5'-CTCCAGTTTCGAGCTTT-3'; HDV primer, 5'-AAGTAGCCCAGGTCGGACC-3'; HP primer, 5'-ACCAGGTAATATACCACAAC-3'.

As shown in Figure 29, specific bands corresponding to full-length precursor RNA and 3' cleavage products were detected from cells transfected with the self-processing cassettes. All three constructs, in addition to being transcriptionally active, appear to self-process efficiently in the cytoplasm of OST7-1 cells. In particular, the HH and HP(GC) constructs self-process to greater than 95%. The overall extent of self-processing in OST7-1 cells appears to be strikingly similar to the extent of self-processing *in vitro* (Figure 29 "In Vitro +MgCl₂" vs. "Cellular").

Consistent with the *in vitro* self-processing results, the HP(GU) cassette self-processed to approximately 50% in OST7-1 cells. As expected, transfection with plasmids containing the HH(mutant) cassette yielded a primer-extension product corresponding to the full-length RNA with no detectable cleavage products (Figure 29). The latter result strongly suggests that the primer extension band corresponding to the 3' cleavage product is not an artifact of reverse transcription.

Applicant was concerned with the possibility that RNA self-processing might occur during cell lysis, RNA isolation and /or the primer extension assay. Two precautions were taken to exclude this possibility. First, 50 mM EDTA was included in the lysis buffer. EDTA is a strong chelator of divalent

metal ions such as Mg^{2+} and Ca^{2+} that are necessary for ribozyme activity. Divalent metal ions are therefore unavailable to self-processing RNAs following cell lysis. A second precaution involved using primers in the primer-extension assay that were designed to hybridize to essential regions of the processing ribozyme. Binding of these primers should prevent the 3' cis-acting ribozymes from folding into the conformation essential for catalytic activity.

Two experiments were carried out to further eliminate the possibility that self-processing is occurring either during RNA preparations or during the primer extension analysis. The first experiment involves primer extension analysis on full-length precursor RNAs that were added to non-transfected OST7-1 lysates after cell lysis. Thus, only if self-processing is occurring at some point after lysis would cleavage products be detected. Full-length precursor RNAs were prepared by transcribing under conditions of low Mg^{2+} (5 mM) and high NTP concentration (total 12 mM) in an attempt to eliminate the free Mg^{2+} required for the self-processing reaction (Michel et al. 1992 *Genes & Dev.* 6, 1373). The full-length precursor RNAs were gel-purified, and a known amount was added to lysates of non-transfected OST7-1 cells. RNA was purified from these lysates and incubated for 1 hr in DEPC-treated water at 37° C prior to the standard primer extension analysis (Figure 29, *in vitro* "-MgCl₂" control). The predominant RNA detected in all cases corresponds to the primer extension product of full-length precursor RNAs. If, instead, the purified RNA containing the full-length precursor is incubated in 10 mM MgCl₂ prior to the primer extension analysis, most or all of the RNA detected by primer extension analysis undergoes cleavage (Figure 29, *in vitro* "+MgCl₂" control). These results indicate that the standard RNA isolation and primer extension protocols used here do not provide a favorable environment for RNA self-processing, even though the RNA in question is inherently able to undergo self-cleavage.

In a second experiment to demonstrate lack of self-processing during work up, internally-labeled precursor RNAs were prepared and added to non-transfected OST7-1 lysates as in the previous control. The internally-labeled precursor RNAs were carried through the RNA purification and primer extension reactions (in the presence of unlabeled primers) and analyzed to determine the extent of self-processing. By this analysis, the

vast majority of the added full-length RNA remained intact during the entire process of RNA isolation and primer extension.

These two control experiments validate the protocols used and support applicant's conclusion that the self-processing reactions catalyzed by HH, HDV and HP(GC) cassettes are occurring in the cytoplasm of OST7-1 cells.

Sequences in figures 23 through 25 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art.

In addition, those in the art will recognize that Applicant provides guidance through the above examples as to how to best design vectors of this invention so that secondary structure of the mRNA allows efficient cleavage by releasing ribozymes. Thus, the specific constructs are not limiting in this invention. Such constructs can be readily tested as described above for such secondary structure, either by computer folding algorithms or empirically. Such constructs will then allow at least 80% completion of release of ribozymes, which can be readily determined as described above or by methods known in the art. That is, any such secondary structure in the RNA does not reduce release of the ribozymes by more than 20%.

IV. Ribozymes Expressed by RNA Polymerase III

Applicant has determined that the level of production of a foreign RNA, using a RNA polymerase III (pol III) based system, can be significantly enhanced by ensuring that the RNA is produced with the 5' terminus and a 3' region of the RNA molecule base-paired together to form a stable intramolecular stem structure. This stem structure is formed by hydrogen bond interactions (either Watson-Crick or non-Watson-Crick) between nucleotides in the 3' region (at least 8 bases) and complementary nucleotides in the 5' terminus of the same RNA molecule.

Although the example provided below involves a type 2 pol III gene unit, a number of other pol III promoter systems can also be used, for example, tRNA (Hall et al., 1982 *Cell* 29, 3-5), 5S RNA (Nielsen et al., 1993, *Nucleic Acids Res.* 21, 3631-3636), adenovirus VA RNA (Fowlkes and Shenk, 1980 *Cell* 22, 405-413), U6 snRNA (Gupta and Reddy, 1990

Nucleic Acids Res. 19, 2073-2075), vault RNA (Kickoefer et al., 1993 *J. Biol. Chem.* 268, 7868-7873), telomerase RNA (Romero and Blackburn, 1991 *Cell* 67, 343-353), and others.

5 The construct described in this invention is able to accumulate RNA to a significantly higher level than other constructs, even those in which 5' and 3' ends are involved in hairpin loops. Using such a construct the level of expression of a foreign RNA can be increased to between 20,000 and 50,000 copies per cell. This makes such constructs, and the vectors encoding such constructs, excellent for use in decoy, therapeutic editing and antisense protocols as well as for ribozyme formation. In addition, the
10 molecules can be used as agonist or antagonist RNAs (affinity RNAs). Generally, applicant believes that the intramolecular base-paired interaction between the 5' terminus and the 3' region of the RNA should be in a double-stranded structure in order to achieve enhanced RNA
15 accumulation.

Thus, in one preferred embodiment the invention features a pol III promoter system (e.g., a type 2 system) used to synthesize a chimeric RNA molecule which includes tRNA sequences and a desired RNA (e.g., a tRNA-based molecule).

20 The following exemplifies this invention with a type 2 pol III promoter and a tRNA gene. Specifically to illustrate the broad invention, the RNA molecule in the following example has an A box and a B box of the type 2 pol III promoter system and has a 5' terminus or region able to base-pair with at least 8 bases of a complementary 3' end or region of the same RNA
25 molecule. This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using other pol III promoter systems and techniques generally known in the art.

30 By "terminus" is meant the terminal bases of an RNA molecule, ending in a 3' hydroxyl or 5' phosphate or 5' cap moiety. By "region" is meant a stretch of bases 5' or 3' from the terminus that are involved in base-paired interactions. It need not be adjacent to the end of the RNA. Applicant has determined that base pairing of at least one end of the RNA molecule with a region not more than about 50 bases, and preferably only 20 bases, from

the other end of the molecule provides a useful molecule able to be expressed at high levels.

By "3' region" is meant a stretch of bases 3' from the terminus that are involved in intramolecular base-paired interaction with complementary nucleotides in the 5' terminus of the same molecule. The 3' region can be designed to include the 3' terminus. The 3' region therefore is ≥ 0 nucleotides from the 3' terminus. For example, in the S35 construct described in the present invention (Fig. 40) the 3' region is one nucleotide from the 3' terminus. In another example, the 3' region is ~ 43 nt from 3' terminus. These examples are not meant to be limiting. Those in the art will recognize that other embodiments can be readily generated using techniques generally known in the art. Generally, it is preferred to have the 3' region within 100 bases of the 3' terminus.

By "tRNA molecule" is meant a type 2 pol III driven RNA molecule that is generally derived from any recognized tRNA gene. Those in the art will recognize that DNA encoding such molecules is readily available and can be modified as desired to alter one or more bases within the DNA encoding the RNA molecule and/or the promoter system. Generally, but not always, such molecules include an A box and a B box that consist of sequences which are well known in the art (and examples of which can be found throughout the literature). These A and B boxes have a certain consensus sequence which is essential for a optimal pol III transcription.

By "chimeric tRNA molecule" is meant a RNA molecule that includes a pol III promoter (type 2) region. A chimeric tRNA molecule, for example, might contain an intramolecular base-paired structure between the 3' region and complementary 5' terminus of the molecule, and includes a foreign RNA sequence at any location within the molecule which does not affect the activity of the type 2 pol III promoter boxes. Thus, such a foreign RNA may be provided at the 3' end of the B box, or may be provided in between the A and the B box, with the B box moved to an appropriate location either within the foreign RNA or another location such that it is effective to provide pol III transcription. In one example, the RNA molecule may include a hammerhead ribozyme with the B box of a type 2 pol III promoter provided in stem II of the ribozyme. In a second example, the B box may be provided in stem IV region of a hairpin ribozyme. A specific example of such RNA molecules is provided below. Those in the art will

recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "desired RNA" molecule is meant any foreign RNA molecule which is useful from a therapeutic, diagnostic, or other viewpoint. Such molecules include antisense RNA molecules, decoy RNA molecules, enzymatic RNA, therapeutic editing RNA and agonist and antagonist RNA.

By "antisense RNA" is meant a non-enzymatic RNA molecule that binds to another RNA (target RNA) by means of RNA-RNA interactions and alters the activity of the target RNA (Eguchi et al., 1991 *Annu. Rev. Biochem.* 60, 631-652). By "enzymatic RNA" is meant an RNA molecule with enzymatic activity (Cech, 1988 *J. American. Med. Assoc.* 260, 3030-3035). Enzymatic nucleic acids (ribozymes) act by first binding to a target RNA. Such binding occurs through the target binding portion of an enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA.

By "decoy RNA" is meant an RNA molecule that mimics the natural binding domain for a ligand. The decoy RNA therefore competes with natural binding target for the binding of a specific ligand. For example, it has been shown that over-expression of HIV trans-activation response (TAR) RNA can act as a "decoy" and efficiently binds HIV *tat* protein, thereby preventing it from binding to TAR sequences encoded in the HIV RNA (Sullenger et al., 1990 *Cell* 63, 601-608). This is meant to be a specific example. Those in the art will recognize that this is but one example, and other embodiments can be readily generated using techniques generally known in the art.

By "therapeutic editing RNA" is meant an antisense RNA that can bind to its cellular target (RNA or DNA) and mediate the modification of a specific base.

By "agonist RNA" is meant an RNA molecule that can bind to protein receptors with high affinity and cause the stimulation of specific cellular pathways.

By "antagonist RNA" is meant an RNA molecule that can bind to cellular proteins and prevent it from performing its normal biological function (for example, see Tsai et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 8864-8868).

- 5 In other aspects, the invention includes vectors encoding RNA molecules as described above, cells including such vectors, methods for producing the desired RNA, and use of the vectors and cells to produce this RNA.

- 10 Thus, the invention features a transcribed non-naturally occurring RNA molecule which includes a desired therapeutic RNA portion and an intramolecular stem formed by base-pairing interactions between a 3' region and complementary nucleotides at the 5' terminus in the RNA. The stem preferably includes at least 8 base pairs, but may have more, for example, 15 or 16 base pairs.

- 15 In preferred embodiments, the 5' terminus of the chimeric tRNA includes a portion of the precursor molecule of the primary tRNA molecule, of which ≥ 8 nucleotides are involved in base-pairing interaction with the 3' region; the chimeric tRNA contains A and B boxes; natural sequences 3' of the B box are deleted, which prevents endogenous RNA processing; the
20 desired RNA molecule is at the 3' end of the B box; the desired RNA molecule is between the A and the B box; the desired RNA molecule includes the B box; the desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA; the molecule has an
25 intramolecular stem resulting from a base-paired interaction between the 5' terminus of the RNA and a complementary 3' region within the same RNA, and includes at least 8 bases; and the 5' terminus is able to base pair with at least 15 bases of the 3' region.

- 30 In most preferred embodiments, the molecule is transcribed by a -RNA polymerase III based promoter system, e.g., a type 2 pol III promoter system; the molecule is a chimeric tRNA, and may have the A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases; DNA vector encoding the RNA molecule of claim 51.

In other related aspects, the invention features an RNA or DNA vector encoding the above RNA molecule, with the portions of the vector encoding the RNA functioning as a RNA pol III promoter; or a cell containing the vector ; or a method to provide a desired RNA molecule in a cell, by
5 introducing the molecule into a cell with an RNA molecule as described above. The cells can be derived from animals, plants or human beings.

In order for RNA-based gene therapy approaches to be effective, sufficient amounts of the therapeutic RNA must accumulate in the appropriate intracellular compartment of the treated cells. Accumulation is
10 a function of both promoter strength of the antiviral gene, and the intracellular stability of the antiviral RNA. Both RNA polymerase II (pol II) and RNA polymerase III (pol III) based expression systems have been used to produce therapeutic RNAs in cells (Sarver & Rossi, 1993 *AIDS Res. & Human Retroviruses* 9, 483-487; Yu et al., 1993 *P.N.A.S.(USA)* 90, 6340-
15 6344). However, pol III based expression cassettes are theoretically more attractive for use in expressing antiviral RNAs for the following reasons. Pol II produces messenger RNAs located exclusively in the cytoplasm, whereas pol III produces functional RNAs found in both the nucleus and the cytoplasm. Pol II promoters tend to be more tissue restricted, whereas pol
20 III genes encode tRNAs and other functional RNAs necessary for basic "housekeeping" functions in all cell types. Therefore, pol III promoters are likely to be expressed in all tissue types. Finally, pol III transcripts from a given gene accumulate to much greater levels in cells relative to pol II genes.

25 Intracellular accumulation of therapeutic RNAs is also dependent on the method of gene transfer used. For example, the retroviral vectors presently used to accomplish stable gene transfer, integrate randomly into the genome of target cells. This random integration leads to varied expression of the transferred gene in individual cells comprising the bulk
30 treated cell population. Therefore, for maximum effectiveness, the transferred gene must have the capacity to express therapeutic amounts of the antiviral RNA in the entire treated cell population, regardless of the integration site.

Pol III System

The following is just one non-limiting example of the invention. A pol III based genetic element derived from a human tRNA^{met} gene and termed $\Delta 3-5$ (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), has been adapted to express antiviral RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523). This element was inserted into the DC retroviral vector (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523) to accomplish stable gene transfer, and used to express antisense RNAs against moloney murine leukemia virus and anti-HIV decoy RNAs (Sullenger et al., 1990 *Mol. Cell. Biol.* 10, 6512-6523; Sullenger et al., 1990 *Cell* 63, 601-608; Sullenger et al., 1991 *J. Virol.* 65, 6811-6816; Lee et al., 1992 *The New Biologist* 4, 66-74). Clonal lines are expanded from individual cells present in the bulk population, and therefore express similar amounts of the therapeutic RNA in all cells. Development of a vector system that generates therapeutic levels of therapeutic RNA in all treated cells would represent a significant advancement in RNA based gene therapy modalities.

Applicant examined hammerhead (HHI) ribozyme (RNA with enzymatic activity) expression in human T cell lines using the $\Delta 3-5$ vector system (These constructs are termed " $\Delta 3-5$ /HHI"; Fig. 34). On average, ribozymes were found to accumulate to less than 100 copies per cell in the bulk T cell populations. In an attempt to improve expression levels of the $\Delta 3-5$ chimera, the applicant made a series of modified $\Delta 3-5$ gene units containing enhanced promoter elements to increase transcription rates, and inserted structural elements to improve the intracellular stability of the ribozyme transcripts (Fig. 34). One of these modified gene units, termed S35, gave rise to more than a 100-fold increase in ribozyme accumulation in bulk T cell populations relative to the original $\Delta 3-5$ /HHI vector system. Ribozyme accumulation in individual clonal lines from the pooled T cell populations ranged from 10 to greater than 100 fold more than those achieved with the original $\Delta 3-5$ /HHI version of this vector.

The S35 gene unit may be used to express other therapeutic RNAs including, but not limited to, ribozymes, antisense, decoy, therapeutic editing, agonist and antagonist RNAs. Application of the S35 gene unit would not be limited to antiviral therapies, but also to other diseases, such as cancer, in which therapeutic RNAs may be effective. The S35 gene unit may be used in the context of other vector systems besides retroviral

vectors, including but not limited to, other stable gene transfer systems such as adeno-associated virus (AAV; Carter, 1992 *Curr. Opin. Genet. Dev.* 3, 74), as well as transient vector systems such as plasmid delivery and adenoviral vectors (Berkner, 1988 *BioTechniques* 6, 616-629).

- 5 As described below, the S35 vector encodes a truncated version of a tRNA wherein the 3' region of the RNA is base-paired to complementary nucleotides at the 5' terminus, which includes the 5' precursor portion that is normally processed off during tRNA maturation. Without being bound by any theory, Applicant believes this feature is important in the level of
10 expression observed. Thus, those in the art can now design equivalent RNA molecules with such high expression levels. Below are provided examples of the methodology by which such vectors and tRNA molecules can be made.

Δ 3-5 Vectors

- 15 The use of a truncated human tRNA^{met} gene, termed Δ 3-5 (Fig. 33; Adeniyi-Jones et al., 1984 *supra*), to drive expression of antisense RNAs, and subsequently decoy RNAs (Sullenger et al., 1990 *supra*) has recently been reported. Because tRNA genes utilize internal pol III promoters, the antisense and decoy RNA sequences were expressed as chimeras
20 containing tRNA^{met} sequences. The truncated tRNA genes were placed into the U3 region of the 3' moloney murine leukemia virus vector LTR (Sullenger et al., 1990 *supra*).

Base-Paired Structures

- Since the Δ 3-5 vector combination has been successfully used to
25 express inhibitory levels of both antisense and decoy RNAs, applicant cloned ribozyme-encoding sequences (termed as " Δ 3-5/HHI") into this vector to explore its utility for expressing therapeutic ribozymes. However, low ribozyme accumulation in human T cell lines stably transduced with this vector was observed (Fig. 35). To try and improve accumulation of the
30 ribozyme, applicant incorporated various RNA structural elements (Fig. 34) into one of the ribozyme chimeras (Δ 3-5/HHI).

Two strategies were used to try and protect the termini of the chimeric transcripts from exonucleolytic degradation. One strategy involved the incorporation of stem-loop structures into the termini of the transcript. Two

such constructs were cloned, S3 which contains a stem-loop structure at the 3' end, and S5 which contains stem-loop structures at both ends of the transcript (Figure 34). The second strategy involved modification of the 3' terminal sequences such that the 5' terminus and the 3' end sequences can form a stable base-paired stem. Two such constructs were made: S35 in which the 3' end was altered to hybridize to the 5' leader and acceptor stem of the tRNA^{met} domain, and S35Plus which was identical to S35 but included more extensive structure formation within the non-ribozyme portion of the $\Delta 3-5$ chimeras (Figure 34). These stem-loop structures are also intended to sequester non-ribozyme sequences in structures that will prevent them from interfering with the catalytic activity of the ribozyme. These constructs were cloned, producer cell lines were generated, and stably-transduced human MT2 (Harada et al., 1985 *supra*) and CEM (Nara & Fischinger, 1988 *supra*) cell lines were established (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). The RNA sequences and structure of S35 and S35 Plus are provided in Figures 40-47.

Referring to Figure 48, there is provided a general structure for a chimeric RNA molecule of this invention. Each N independently represents none or a number of bases which may or may not be base paired. The A and B boxes are optional and can be any known A or B box, or a consensus sequence as exemplified in the figure. The desired nucleic acid to be expressed can be any location in the molecule, but preferably is on those places shown adjacent to or between the A and B boxes (designated by arrows). Figure 49 shows one example of such a structure in which a desired RNA is provided 3' of the intramolecular stem. A specific example of such a construct is provided in Figures 50 and 51.

Example 26: Cloning of $\Delta 3-5$ -Ribozyme Chimera

Oligonucleotides encoding the S35 insert that overlap by at least 15 nucleotides were designed (5' GATCCACTCTGCTGTTCTGTTTTTGA 3' and 5' CGCGTCAAAAACAGAACAGCAGAGTG 3'). The oligonucleotides (10 μ M each) were denatured by boiling for 5 min in a buffer containing 40 mM Tris.HCl, pH8.0. The oligonucleotides were allowed to anneal by snap cooling on ice for 10-15 min.

The annealed oligonucleotide mixture was converted into a double-stranded molecule using Sequenase[®] enzyme (US Biochemicals) in a

buffer containing 40 mM Tris.HCl, pH7.5, 20 mM MgCl₂, 50 mM NaCl, 0.5 mM each of the four deoxyribonucleotide triphosphates, 10 mM DTT. The reaction was allowed to proceed at 37°C for 30 min. The reaction was stopped by heating to 70°C for 15 min.

- 5 The double stranded DNA was digested with appropriate restriction endonucleases (*Bam*HI and *Mlu*I) to generate ends that were suitable for cloning into the Δ3-5 vector.

- 10 The double-stranded insert DNA was ligated to the Δ3-5 vector DNA by incubating at room temperature (about 20°C) for 60 min in a buffer containing 66 mM Tris.HCl, pH 7.6, 6.6 mM MgCl₂, 10 mM DTT, 0.066 μM ATP and 0.1U/μl T4 DNA Ligase (US Biochemicals).

- 15 Competent *E. coli* bacterial strain was transformed with the recombinant vector DNA by mixing the cells and DNA on ice for 60 min. The mixture was heat-shocked by heating to 37°C for 1 min. The reaction mixture was diluted with LB media and the cells were allowed to recover for 60 min at 37°C. The cells were plated on LB agar plates and incubated at 37°C for ~ 18 h.

- 20 Plasmid DNA was isolated from an overnight culture of recombinant clones using standard protocols (Ausubel et al., *Curr. Protocols Mol. Biology* 1990, Wiley & Sons, NY).

The identity of the clones were determined by sequencing the plasmid DNA using the Sequenase[®] DNA sequencing kit (US Biochemicals).

- 25 The resulting recombinant Δ3-5 vector contains the S35 sequence. The HHI encoding DNA was cloned into this Δ3-5-S35 containing vector using *Sac*II and *Bam*HI restriction sites.

Example 27: Northern analysis

- 30 RNA from the transduced MT2 cells were extracted and the presence of Δ3-5/ribozyme chimeric transcripts were assayed by Northern analysis (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY). Northern analysis of RNA extracted from MT2 transductants showed that Δ3-5/ribozyme chimeras of appropriate sizes were expressed (Fig. 35,36). In addition, these results demonstrated the relative differences in accumulation among the different constructs (Figure 35,36). The pattern of

expression seen from the $\Delta 3$ -5/HHI ribozyme chimera was similar to 12 other ribozymes cloned into the $\Delta 3$ -5 vector (not shown). In MT-2 cell line, $\Delta 3$ -5/HHI ribozyme chimeras accumulated, on average, to less than 100 copies per cell.

- 5 Addition of a stem-loop onto the 3' end of $\Delta 3$ -5/HHI did not lead to increased $\Delta 3$ -5 levels (S3 in Fig. 35,36). The S5 construct containing both 5' and 3' stem-loop structures also did not lead to increased ribozyme levels (Fig. 35,36).

- 10 Interestingly, the S35 construct expression in MT2 cells was about 100-fold more abundant relative to the original $\Delta 3$ -5/HHI vector transcripts (Fig. 35,36). This may be due to increased stability of the S35 transcript.

Example 28: Cleavage activity

- 15 To assay whether ribozymes transcribed in the transduced cells contained cleavage activity, total RNA extracted from the transduced MT2 T cells were incubated with a labeled substrate containing the HHI cleavage site (Figure 37). Ribozyme activity in all but the S35 constructs, was too low to detect. However, ribozyme activity was detectable in S35-transduced T cell RNA. Comparison of the activity observed in the S35-transduced MT2 RNA with that seen with MT2 RNA in which varying
- 20 amounts of in vitro transcribed S5 ribozyme chimeras, indicated that between 1-3 nM of S35 ribozyme was present in S35-transduced MT2 RNA. This level of activity corresponds to an intracellular concentration of 5,000-15,000 ribozyme molecules per cell.

Example 29: Clonal variation

- 25 Variation in the ribozyme expression levels among cells making up the bulk population was determined by generating several clonal cell lines from the bulk S35 transduced CEM line (*Curr. Protocols Mol. Biol.* 1992, ed. Ausubel et al., Wiley & Sons, NY) and the ribozyme expression and activity levels in the individual clones were measured (Figure 38 and 39).
- 30 All the individual clones were found to express active ribozyme. The ribozyme activity detected from each clone correlated well with the relative amounts of ribozyme observed by Northern analysis. Steady state ribozyme levels among the clones ranged from approximately 1,000 molecules per cell in clone G to 11,000 molecules per cell in clone H (Fig.

38). The mean accumulation among the clones, calculated by averaging the ribozyme levels of the clones, exactly equaled the level measured in the parent bulk population. This suggests that the individual clones are representative of the variation present in the bulk population.

- 5 The fact that all 14 clones were found to express ribozyme indicate that the percentage of cells in the bulk population expressing ribozyme is also very high. In addition, the lowest level of expression in the clones was still more than 10-fold that seen in bulk cells transduced with the original Δ3-5 vector. Therefore, the S35 gene unit should be much more effective
- 10 in a gene therapy setting in which bulk cells are removed, transduced and then reintroduced back into a patient.

Example 30: Stability

- Finally, the bulk S35-transduced line, resistant to G418, was propagated for a period of 3 months (in the absence of G418) to determine
- 15 If ribozyme expression was stable over extended periods of time. This situation mimicks that found in the clinic in which bulk cells are transduced and then reintroduced into the patient and allowed to propagate. There was a modest 30% reduction of ribozyme expression after 3 months. This difference probably arose from cells with varying amount of ribozyme
- 20 expression and exhibiting different growth rates in the culture becoming slightly more prevalent in the culture. However, ribozyme expression is apparently stable for at least this period of time.

Example 31: Design and construction of TRZ-tRNA Chimera

- A transcription unit, termed TRZ, is designed that contains the S35
- 25 motif (Figure 52). A desired RNA (e.g. ribozyme) can be inserted into the indicated region of TRZ tRNA chimera. This construct might provide additional stability to the desired RNA. TRZ-A and TRZ-B are non-limiting examples of the TRZ-tRNA chimera.

- Referring to Fig. 53-54, a hammerhead ribozyme targeted to site I
- 30 (HHITRZ-A; Fig. 53) and a hairpin ribozyme (HPITRZ-A; Fig. 54), also targeted to site I, is cloned individually into the indicated region of TRZ tRNA chimera. The resulting ribozyme transcripts retain full RNA cleavage activity (see for example Fig. 55). Applicant has shown that efficient

expression of these TRZ tRNA chimera can be achieved in mammalian cells.

Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated region of TRZ-tRNA chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in Figures 40-47 and 50 - 54 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and deletions) of the above examples can be readily generated using techniques known in the art, are within the scope of the present invention.

Example 32: Ribozyme expression in T cell lines

Ribozyme expression in T cell lines stably-transduced with either a retroviral-based or an Adeno-associated virus (AAV)-based ribozyme expression vector (Figure 56). The human T cell lines MT2 and CEM were transduced with either retroviral or AAV vectors encoding a neomycin selectable marker and a ribozyme (S35/HHI) expressed from pol III met; tRNA-driven promoter. Cells stably-transduced with the vectors were selectively expanded in medium containing the neomycin antibiotic derivative, G418 (0.7 mg/ml). Ribozyme expression in the stable cell lines was then analyzed by Northern analysis. The probe used to detect ribozyme transcripts also cross-hybridized with human met; tRNA sequences. Referring to Figure 56, S35/HHI RNA accumulates to significant levels in MT2 and CEM cells when transduced with either the retrovirus or the AAV vector.

These are meant to be non-limiting examples, those skilled in the art will recognize that other vectors such as adenovirus vector (Figure 57), plasmid DNA vector, alpha virus vectors and the other derivatives thereof, can be readily generated to deliver the desired RNA, using techniques known in the art and are within the scope of this invention. Additionally, the transcription units can be expressed individually or in multiples using pol II and/or pol III promoters.

References cited herein, as well as Draper WO 93/23569, 94/02495, 94/06331, Sullenger WO 93/12657, Thompson WO 93/04573, and Sullivan

WO 94/04609, and 93/11253 describe methods for use of vectors described herein, and are incorporated by reference herein. In particular these vectors are useful for administration of antisense and decoy RNA molecules.

5 Example 33: Ligated Ribozymes are catalytically active

The ability of ribozymes generated by ligation methods, described in Draper et al., PCT WO 93/23569, to cleave target RNA was tested on either matched substrate RNA (Fig. 58) or long (622 nt) RNA (Fig. 59, 60 and 61).

10 Matched substrate RNAs were chemically synthesized using solid-phase RNA synthesis chemistry (Scaringe et al., 1990 Nucleic Acids Res. 18, 5433-5441). Substrate RNA was 5' end-labeled using [γ - 32 P] ATP and polynucleotide kinase (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). Ribozyme reactions were carried out under ribozyme excess conditions (k_{cat}/K_M ; Herschlag and Cech, 1990 Biochemistry 29, 15 10159-10171). Briefly, ribozyme and substrate RNA were denatured and renatured separately by heating to 90°C and snap cooling on ice for 10 min in a buffer containing 50 mM Tris. HCl pH 7.5 and 10 mM MgCl₂. Cleavage reaction was initiated by mixing the ribozyme with the substrate at 37°C. Aliquots of 5 μ l were taken at regular intervals of time and the 20 reaction was stopped by mixing with equal volume of formamide gel loading buffer (Curr. Protocols Mol. Biol. 1992, ed. Ausubel et al., Wiley & Sons, NY). The samples were resolved on 20 % polyacrylamide-urea gel. Referring to Fig. 58, $-\Delta G$ refers to the free energy of binding calculated for base-paired interactions between the ribozyme and the substrate RNA 25 (Turner and Sugimoto, 1988 Supra). RPI A is a HH ribozyme with 6/6 binding arms. This ribozyme was synthesized chemically either as a one piece ribozyme or was synthesized in two fragments followed by ligation to generate a one piece ribozyme. The k_{cat}/K_M values for the two ribozymes were comparable.

30 A template containing T7 RNA polymerase promoter upstream of 622 nt long target sequence, was PCR amplified from a DNA clone. The target RNA (containing HH ribozyme cleavage sites B, C and D) was transcribed from this PCR amplified template using T7 RNA polymerase. The transcript was internally labeled during transcription by including [α - 32 P] CTP as one 35 of the four ribonucleotide triphosphates. The transcription mixture was

5 treated with DNase-1, following transcription at 37°C for 2 hours, to digest away the DNA template used in the transcription. RNA was precipitated with isopropanol and the pellet was washed two times with 70% ethanol to get rid of salt and nucleotides used in the transcription reaction. RNA is resuspended in DEPC-treated water and stored at 4°C. Ribozyme cleavage reactions were carried out under ribozyme excess (k_{cat}/K_M) conditions [Herschlag and Cech 1990 supra]. Briefly, 1000 nM ribozyme and 10 nM internally labeled target RNA were denatured separately by heating to 90°C for 2 min in the presence of 50 mM Tris.HCl, pH 7.5 and 10 mM MgCl₂. The RNAs were renatured by cooling to 37°C for 10-20 min. Cleavage reaction was initiated by mixing the ribozyme and target RNA at 37°C. Aliquots of 5 µl were taken at regular intervals of time and the reaction was quenched by adding equal volume of stop buffer. The samples were resolved on a sequencing gel.

15 Example 34: Hammerhead ribozymes with ≥ 2 base-paired stem II are catalytically active

To decrease the cost of chemical synthesis of RNA, applicant was interested in determining whether the length of stem II region of a typical hammerhead ribozyme (≥ 4 bp stem II) can be shortened without decreasing the catalytic efficiency of the HH ribozyme. The length of stem II was systematically shortened by one base-pair at a time. HH ribozymes with three and two base-paired stem II were chemically synthesized using solid-phase RNA phosphoramidite chemistry (Scaringe et al., 1990 supra).

25 Matched and long substrate RNAs were synthesized and ribozyme assays were carried out as described in example 33. Referring to figures 62, 63 and 64, data shows that shortening stem II of a hammerhead ribozyme does not significantly alter the catalytic efficiency. It is applicant's opinion that hammerhead ribozymes with ≥ 2 base-paired stem II region are catalytically active.

30 Example 35: Synthesis of catalytically active hairpin ribozymes

RNA molecules were chemically synthesized having the nucleotide base sequence shown in Fig. 65 for both the 5' and 3' fragments. The 3' fragments are phosphorylated and ligated to the 5' fragment essentially as described in example 37. As is evident from the Figure 65, the 3' and 5' fragments can hybridize together at helix 4 and are covalently linked via

GAAA sequence. When this structure hybridizes to a substrate, a ribozyme-substrate complex structure is formed. While helix 4 is shown as 3 base pairs it may be formed with only 1 or 2 base pairs.

40 nM mixtures of ligated ribozymes were incubated with 1-5 nM 5' end-labeled matched substrates (chemically synthesized by solid-phase synthesis using RNA phosphoramidite chemistry) for different times in 50 mM Tris/HCl pH 7.5, 10 mM MgCl₂ and shown to cleave the substrate efficiently (Fig. 66).

The target and the ribozyme sequences shown in Fig. 62 and 65 are meant to be non-limiting examples. Those in the art will recognize that other embodiments can be readily generated using other sequences and techniques generally known in the art.

V. Constructs of Hairpin Ribozymes

There follows an improved trans-cleaving hairpin ribozyme in which a new helix (*i.e.*, a sequence able to form a double-stranded region with another single-stranded nucleic acid) is provided in the ribozyme to base-pair with a 5' region of a separate substrate nucleic acid. This helix is provided at the 3' end of the ribozyme after helix 3 as shown in Figure 3. In addition, at least two extra bases may be provided in helix 2 and a portion of the substrate corresponding to helix 2 may be either directly linked to the 5' portion able to hydrogen bond to the 3' end of the hairpin or may have a linker of at least one base. By trans-cleaving is meant that the ribozyme is able to act in *trans* to cleave another RNA molecule which is not covalently linked to the ribozyme itself. Thus, the ribozyme is not able to act on itself in an intramolecular cleavage reaction.

By "base-pair" is meant a nucleic acid that can form hydrogen bond(s) with other RNA sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of interactions.

The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) has several advantages. These include improved stability of the ribozyme-target complex *in vivo*. In addition, an increase in the recognition sequence of the hairpin ribozyme improves the specificity of the ribozyme. This also makes possible the targeting of potential hairpin

ribozyme sites that would otherwise be inaccessible due to neighboring secondary structure.

5 The increase in length of helix 2 of a hairpin ribozyme (with or without helix 5) enhances *trans*-ligation reaction catalyzed by the ribozyme. *Trans*-ligation reactions catalyzed by the regular hairpin ribozyme (4 bp helix 2) is very inefficient (Komatsu *et al.*, 1993 *Nucleic Acids Res.* 21, 185). This is attributed to weak base-pairing interactions between substrate RNAs and the ribozyme. By increasing the length of helix 2 (with or without helix 5) the rate of ligation (*in vitro* and *in vivo*) can be enhanced several fold.

10 Results of experiments suggest that the length of H2 can be 6 bp without significantly reducing the activity of the hairpin ribozyme. The H2 arm length variation does not appear to be sequence dependent. HP ribozymes with 6 bp H2 have been designed against five different target RNAs and all five ribozymes efficiently cleaved their cognate target RNA.
15 Additionally, two of these ribozymes were able to successfully inhibit gene expression (e.g., TNF- α) in mammalian cells. Results of these experiments are shown below.

HP ribozymes with 7 and 8 bp H2 are also capable of cleaving target RNA in a sequence-specific manner, however, the rate of the cleavage
20 reaction is lower than those catalyzed by HP ribozymes with 6 bp H2.

Example 36: 4 and 6 base pair H2

Referring to Figures 67-72, HP ribozymes were synthesized as described above and tested for activity. Surprisingly, those with 6 base pairs in H2 were still as active as those with 4 base pairs.

25 VI. Chemical Modification

Oligonucleotides with 5'-C-alkyl Group

The introduction of an alkyl group at the 5'-position of a nucleoside or nucleotide sugar introduces an additional center of chirality into the sugar moiety. Referring to Fig. 75, the general structures of 5'-C-alkylnucleotides
30 belonging to the D-allose, 2, and L-talose, 3, sugar families are shown. The family names are derived from the known sugars D-allose and L-talose ($R_1 = CH_3$ in 2 and 3 in Figure 75). Useful specific D-allose and L-talose

nucleotide derivatives are shown in Figure 76, 29-32 and Figure 77, 58-61 respectively.

This invention relates to the use of 5'-C-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 5'-C-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 5'-C-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 5'-C-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 5'-C-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 5'-C-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 5'-C-alkylnucleosides, that is a nucleotide base having at the 5'-position on the sugar molecule an alkyl moiety. In a related aspect, the invention also features 5'-C-alkylnucleotides, and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably

includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above. In preferred embodiments, the sugar of the nucleoside or nucleotide is in an optically pure form, as the talose or allose sugar.

- 5 Examples of various alkyl groups useful in this invention are shown in Figure 75, where each R_1 group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More
10 preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, $=O$, $=S$, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one
15 carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy,
20 $=O$, $=S$, NO_2 , halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons,
25 more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, $=O$, $=S$, NO_2 or $N(CH_3)_2$, amino or SH.

- Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an
30 aromatic group which has at least one ring having a conjugated π electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an
35 alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring

atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

- 10 In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 5'-C-alkylnucleotides; *e.g.* enzymatic nucleic acids having a 5'-C-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic
- 15 molecule with at least one nucleotide having at its 5'-position an alkyl group. In other related aspects, the invention features 5'-C-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

- 20 The 5'-C-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

- 25 In another aspect, the invention features a method for conversion of a protected allo sugar to a protected talo sugar. In the method, the protected allo sugar is contacted with triphenyl phosphine, diethylazodicarboxylate, and *p*-nitrobenzoic acid under inversion causing conditions to provide the protected talo sugar. While one example of such conditions is provided
- 30 below, those in the art will recognize other such conditions. Applicant has found that such conversion allows for ready synthesis of all types of nucleotide bases as exemplified in the figures.

- While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particularly useful for
- 35 enzymatic RNA molecules. Thus, below is provided examples of such

molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 5'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides shown in Figure 75 are possible.

The following are non-limiting examples showing the synthesis of nucleic acids using 5'-C-alkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 37: Synthesis of Hammerhead Ribozymes Containing 5'-C-Alkyl-nucleotides & Other Modified Nucleotides

The method of synthesis would follow the procedure for normal RNA synthesis as described in Usman,N.; Ogilvie,K.K.; Jiang,M.-Y.; Cedergren,R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe,S.A.; Franklyn,C.; Usman,N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 26-29 and 56-59). These 5'-C-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 38: Methyl-2,3-O-Isopropylidene-6-Deoxy-β-D-allofuranoside (4)

A suspension of L-rhamnose (100 g, 0.55 mol), CuSO₄ (120 g) and conc. H₂SO₄ (4.0 mL) in 1.0 L of dry acetone was mixed for 24 h at RT, then filtered. Conc. NH₄OH (5 mL) was added to the filtrate and the newly formed precipitate was filtered. The residue was concentrated *in vacuo*, coevaporated with pyridine (2 x 300 mL), dissolved in pyridine (500 mL) and cooled to 0 °C. A solution of *p*-toluenesulfonylchloride (107 g, 0.56

mmol) in dry DCE (500 mL) was added dropwise over 0.5 h. The reaction mixture was left for 16 h at RT. The reaction was quenched by adding ice-water (0.5 L) and, after mixing for 0.5 h, was extracted with chloroform (0.75 L). The organic layer was washed with H₂O (2 x 500 mL), 10% H₂SO₄ (2 x 300 mL), water (2 x 300 mL), sat. NaHCO₃ (2 x 300 mL), brine (2 x 300 mL), dried over MgSO₄ and evaporated to dryness. The residue (115 g) was dissolved in dry MeOH (1 L) and treated with NaOMe (23.2 g, 0.42 mmol) in MeOH. The reaction mixture was left for 16 h at 20 °C, neutralized with dry CO₂ and evaporated to dryness. The residue was suspended in chloroform (750 mL), filtered, concentrated to 100 mL and purified by flash chromatography in CHCl₃ to yield 45 g (37%) of compound 4.

Example 39: Methyl-2,3-O-Isopropylidene-5-O-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (5).

To solution of methylfuranoside 4 (12.5 g 62.2 mmol) and AgNO₃ (21.25 g, 125.0 mmol) in dry DMF (300 mL) *t*-butyldiphenylsilyl chloride (22.2 g, 81 mmol) was added dropwise under Ar over 0.5 h. The reaction mixture was stirred for 4 h at RT, diluted with CHCl₃ (200 mL), filtered and evaporated to dryness (below 40 °C using a high vacuum oil pump). The residue was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The residue was purified by flash chromatography in CH₂Cl₂ to yield 20.0 g (75%) of compound 5.

Example 40: Methyl-5-O-*t*-Butyldiphenylsilyl-6-Deoxy-β-D-Allofuranoside (6).

Methylfuranoside 5 (13.5 g, 30.6 mmol) was dissolved in CF₃COOH:dioxane:H₂O / 2:1:1 (v/v/v, 200 mL) and stirred at 24 °C for 45 m. The reaction mixture was cooled to -10 °C, neutralized with conc. NH₄OH (140 mL) and extracted with CH₂Cl₂ (500 mL). The organic layer was separated, washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL), dried over MgSO₄ and evaporated to dryness. The product 6 was purified by flash chromatography using a 0-10% MeOH gradient in CH₂Cl₂. Yield 9.0 g (76%).

Example 41: Methyl-2,3-di-O-Benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy- β -D-Allofuranoside (7).

Methylfuranoside 6 (7.0 g, 17.5 mmol) was coevaporated with pyridine (2 x 100 mL) and dissolved in pyridine (100 mL). Benzoyl chloride (5.4 g, 38.5 mmol) was added and the reaction mixture was left at RT for 16 h. Dry EtOH (50 mL) was added and the reaction mixture was evaporated to dryness after 0.5 h. The residue was dissolved in CH₂Cl₂ (300 mL), washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL) dried over MgSO₄ and evaporated to dryness. The product was purified by flash chromatography in CH₂Cl₂ to yield 9.5 g (89%) of compound 7.

Example 42: 1-O-Acetyl-2,3-di-O-benzoyl-5-O-t-Butyldiphenylsilyl-6-Deoxy- β -D-Allofuranose (8).

Dibenzoate 7 (4.7 g, 7.7 mmol) was dissolved in a mixture of AcOH (10.0 mL), Ac₂O (20.0 mL) and EtOAc (30 mL) and the reaction mixture was cooled 0 °C. 98% H₂SO₄ (0.15 mL) was then added. The reaction mixture was kept at 0 °C for 16 h, and then poured into a cold 1:1 mixture of sat. NaHCO₃ and EtOAc (150 mL). After 0.5 h of vigorous stirring the organic phase was separated, washed with brine (2 x 75 mL), dried over MgSO₄, evaporated to dryness and coevaporated with toluene (2 x 50 mL). The product was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 4.0 g (82% as a mixture of α and β isomers).

Example 43: 1-(2',3'-di-O-Benzoyl-5'-O-t-Butyldiphenylsilyl-6'-Deoxy- β -D-Allofuranosyl)uracil (9).

Uracil (1.44 g, 11.5 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT, evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (6.36 g, 10.0 mmol) in dry CH₃CN (100 mL), followed by CF₃SO₃SiMe₃ (2.8 g, 12.6 mmol). The reaction mixture was kept at 24 °C for 16 h, concentrated to 1/3 of its original volume, diluted with 100 mL of CH₂Cl₂ and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄, and evaporated to dryness. The product 9 was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 5.7 g (80%).

Example 44: *N*⁴-Benzoyl-1-(2',3'-Di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)Cytosine (10).

*N*⁴-benzoylcytosine (1.84 g, 8.56 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (3 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL), followed by CF₃SO₃SiMe₃ (4.76 g, 21.4 mmol). The reaction mixture was boiled under reflux for 5 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 1.8 g (55%) of compound 10.

Example 45: *N*⁶-Benzoyl-9-(2',3'-di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)adenine (11).

*N*⁶-benzoyladenine (2.86 g, 11.86 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (7 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a solution of acetates 8 (3.6 g, 5.6 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.59 g, 29.7 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL), brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product 11 was purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂. Yield: 2.7 g (60%).

Example 46: *N*²-Isobutyryl-9-(2',3'-di-*O*-Benzoyl-5'-*O*-*t*-Butyldiphenylsilyl-6'-Deoxy-β-D-Allofuranosyl)guanine (12).

*N*²-isobutyrylguanine (1.47 g, 11.2 mmol) was suspended in mixture of hexamethyldisilazane (100 mL) and pyridine (50 mL) and boiled under reflux until complete dissolution (6 h) occurred, and then for an additional hour. The reaction mixture was cooled to RT evaporated to dryness and coevaporated with dry toluene (2 x 50 mL). To the residue was added a

solution of acetates 8 (3.4 g, 5.3 mmol) in dry CH₃CN (100 mL) followed by CF₃SO₃SiMe₃ (6.22 g, 28.0 mmol). The reaction mixture was boiled under reflux for 8 h, cooled to RT, concentrated to 1/3 of its original volume, diluted with CH₂Cl₂ (100 mL) and extracted with sat. NaHCO₃ (2 x 50 mL),
5 brine (2 x 50 mL) dried over MgSO₄ and evaporated to dryness. The product 12 was purified by flash chromatography using a gradient of 0-2% MeOH in CH₂Cl₂. Yield: 2.1g (54%).

Example 47: N⁶-Benzoyl-9-(2',3'-di-O-benzoyl-6'-Deoxy-β-D-Allofuranosyl)adenine (15).

- 10 Nucleoside 11 (1.65 g, 2.0 mmol) was dissolved in THF (50 mL) and a 1 M solution of TBAF in THF (4 mL) was added. The reaction mixture was kept at RT for 4 h, evaporated to dryness and the product purified by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ to yield 1.0 g (85%) of compound 15.

- 15 Example 48: N⁶-Benzoyl-9-(2',3'-di-O-Benzoyl-5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)-adenine (19).

- 20 Nucleoside 15 (0.55 g, 0.92 mmol) was dissolved in dry CH₂Cl₂ (50 mL). AgNO₃ (0.34 g, 2.0 mmol), dimethoxytrityl chloride (0.68 g, 2.0 mmol) and sym-collidine (0.48 g) were added under Ar. The reaction mixture was stirred for 2h, diluted with CH₂Cl₂ (100 mL), filtered, evaporated to dryness and coevaporated with toluene (2 x 50 mL). Purification by flash chromatography using a gradient of 0-5% MeOH in CH₂Cl₂ yielded 0.8 g (97%) of compound 19.

- 25 Example 49: N⁶-Benzoyl-9-(-5'-O-Dimethoxytrityl-6'-Deoxy-β-D-Allofuranosyl)adenine (23).

- 30 Nucleoside 19 (1.8 g, 2 mmol) was dissolved in dioxane (50 mL), cooled to 0 °C and 2 M NaOH (50 mL) was added. The reaction mixture was kept at 0 °C for 45 m, neutralized with Dowex 50 (Pyr⁺ form), filtered and the resin was washed with MeOH (2 x 50 mL). The filtrate was then evaporated to dryness. Purification by flash chromatography using a gradient of 0-10% MeOH in CH₂Cl₂ yielded 1.1 g (80%) of 23.

Example 50: Λ^6 -Benzoyl-9-(-5'-O-Dimethoxytrityl-2'-O-*t*-butyldimethylsilyl-6'-Deoxy- β -D-Allofuranosyl)adenine (27).

Nucleoside 23 (1.2 g, 1.8 mmol) was dissolved in dry THF (50 mL). Pyridine (0.50 g, 8 mmol) and AgNO₃ (0.4 g, 2.3 mmol) were added. After the AgNO₃ dissolved (1.5 h), *t*-butyldimethylsilyl chloride (0.35 g, 2.3 mmol) was added and the reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with CH₂Cl₂ (100 mL), filtered into sat. NaHCO₃ (50 mL), extracted, the organic layer washed with brine (2 x 50 mL), dried over MgSO₄ and evaporated to dryness. The product 27 was purified by flash chromatography using a hexanes:EtOAc / 7:3 gradient. Yield: 0.7 g (50%).

Example 51: Λ^6 -Benzoyl-9-(-5'-O-Dimethoxytrityl-2'-O-*t*-butyldimethylsilyl-6'-Deoxy- β -D-Allofuranosyl)adenine-3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31).

Standard phosphitylation of 27 according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidite 31 in 73% yield.

Example 52: Methyl-5-O-*p*-Nitrobenzoyl-2,3-O-Isopropylidene-6-deoxy- β -L-Talofuranoside (5)

Methylfuranoside 4 (3.1 g 14.2 mmol) was dissolved in dry dioxane (200 mL), *p*-nitrobenzoic acid (10.0 g, 60 mmol) and triphenylphosphine (15.74 g, 60.0 mmol) were added followed by DEAD (10.45 g, 60.0 mmol). The reaction mixture was left at RT for 16 h, EtOH (5 mL) was added, and after 0.5 h the reaction mixture was evaporated to dryness. The residue was dissolved in CH₂Cl₂ (300 mL) washed with sat. NaHCO₃ (2 x 75 mL), brine (2 x 75 mL) dried over MgSO₄ and evaporated to dryness. Purification by flash chromatography using a hexanes:EtOAc / 9:1 gradient yielded 4.1 g (78%) of compound 33. Subsequent debenzoylation (NaOMe/MeOH) and silylation (see preparation of 5) led to L-talofuranoside 34 which was converted to phosphoramidites 58-61 using the same methodology as described above for the preparation of the phosphoramidites of the D-allo-isomers 29-32.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage

or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan et al., PCT WO 94/ 02595.

- 5 The ribozymes and the target RNA containing site O were synthesized, deprotected and purified as described above. RNA cleavage assay was carried out at 37°C in the presence of 10 mM MgCl₂ as described above.

Applicant has substituted 5'-C-Me-L-talo nucleotides at positions A6,
10 A9, A9 + G10, C11.1 and C11.1 + G10, as shown in Figure 78 (HH-O1 to HH-O5). HH-O 1,2,4 and 5 showed almost wild type activity (Figure 79). However, HH-O3 demonstrated low catalytic activity. Ribozymes HH-O1, 2, 3, 4 and 5 are also extremely resistant to degradation by human serum nucleases.

15 Oligonucleotides with 2'-Deoxy-2'-Alkyl nucleotide

This invention uses 2'-deoxy-2'-alkyl nucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkyl nucleotide-containing enzymatic
20 nucleic acids are catalytic nucleic molecules that contain 2'-deoxy-2'-alkyl nucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in
25 a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkyl nucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides.
30 Contrary to the findings of De Mesmaeker *et al.* applicant has found that such nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide
35 containing this modification, if that moiety is not in an essential base pair

forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

Thus, in one aspect, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 81, where each R group is any alkyl. The term "alkyl" does not include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides (preferably not a 2'-alkyl- uridine or thymidine); *e.g.* enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall

activity *in vivo*. Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

5 In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 80 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 80, and the binding arms correspond to the bases from the 3'-end to base 15.1, and 10 from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra*.

15 Figure 80 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. This is not to be taken as an indication that the Figure is prior art to the pending claims, or that the art discussed is prior art to those claims. Referring to Figure 80 the preferred sequence of a hammerhead ribozyme 20 in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 81 are 25 possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al. Biochemistry* 1992, 31, 5005-5009 and Paoletta *et al., EMBO J.* 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

30 Ribozymes from Figure 80 and Table 45 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table 45, all 2'-modified ribozymes showed very large and significant increases in stability in human serum 35 (shown) and in the other fluids described below (Example 55, data not shown). The order of most aggressive nuclease activity was fetal bovine

serum, > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio B was calculated (Table 45). This B value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in overall stability and activity. These increases in B indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 81 also increased the $t_{1/2}$ of the resulting modified ribozymes. However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 86 compound 37 may be used as a general intermediate to prepare derivatized 2'-C-alkyl phosphoramidites, where X is CH₃, or an alkyl, or other group described above.

The following are non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance.

Example 53: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkyl nucleotides & Other 2'-Modified Nucleotides

The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cedergren, R.J. *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 10, 12, 17, 22, 31, 18, 26, 32, 36 and 38). Other 2'-modified phosphoramidites were prepared according to: 3 & 4, Eckstein *et al.* *International Publication* No. WO 92/07065; and 5 Kois *et al.* *Nucleosides & Nucleotides* 1993, 12, 1093-1109. The average stepwise coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 80. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense

oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 54: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations were ~ 1 nM. Total reaction volumes were 50 mL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were initiated by mixing substrate and ribozyme solutions at t = 0. Aliquots of 5 mL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time point was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis. Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 55: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated in ethanol and pelleted by centrifugation. Each pellet was resuspended in 20 mL of appropriate fluid (human serum, human plasma, human synovial fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The samples were placed into a 37 °C incubator and 2 mL aliquots were withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m. Aliquots were added to 20 mL of a solution containing 95% formamide and 0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further nuclease activity and the samples were frozen until loading onto gels. Ribozymes were size-fractionated by electrophoresis in 20% acrylamide/8M urea gels. The amount of intact ribozyme at each time point was quantified by scanning the bands with a phosphorimager (Molecular Dynamics) and the half-life of each ribozyme in the fluids was determined by plotting the percent intact ribozyme vs the time of incubation and extrapolation from the graph.

Example 56: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid*

Chemistry, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylamino-pyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance
5 of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

10 Example 57: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl -Uridine (8)

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, 7, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide
15 (0.5 g) was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product 8 purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 58: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

20 A solution of 8 (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with
25 chloroform:methanol / 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched
30 with methanol (20 mL), evaporated, dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of 9 as a white foam.

Example 59: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (10)

5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. N,N-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

Example 60: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-N⁴-Acetyl-Cytidine (11)

15 Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed *in vacuo*. The resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH₄OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by coevaporation with anhydrous pyridine and acetylated with acetic anhydride (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as a white foam.

Example 61: 5'-O-Dimethoxytrityl-2'-C-Allyl-N⁴-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 62: 5'-O-Dimethoxytrityl-2'-C-allyl-N⁴-Acetyl-Cytidine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N⁴-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. N,N-Diisopropylethylamine (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 63: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine 14 (Hansske, F.; Madej, D.; Robins, M. J. *Tetrahedron* 1984, 40, 125 and Matsuda, A.; Takenuki, K.; Tanaka, S.; Sasaki, T.; Ueda, T. *J. Med. Chem.* 1991, 34, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH₂Cl₂.

Example 64: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 65: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH₂Cl₂: MeOH / 15:1)

Example 66: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine 14 (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 67: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH₂Cl₂.

Example 68: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16)

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture

- was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and
- 5 purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-difluoromethylene-uridine 16 (1.05 g, 1.8 mmol, 45%).

Example 69: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (18)

- 10 1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture
- 15 was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂: MeOH / 15:1).

20 Example 70: 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine 20

- Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl₃ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) uridine 19 (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was
- 25 added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved
- 30 in dioxane (10 mL) and aq. ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was concentrated *in vacuo*, dissolved in
- 35 CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The

organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

5 Example 71: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine 21

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 20 (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 21 (0.88 g, 1.5 mmol, 75%).

Example 72: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine 3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidite) (22)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetyl-cytosine 21 (0.88 g, 1.5 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product 22 (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.36 (CH₂Cl₂:MeOH / 20:1).

Example 73: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyl
disiloxane-1,3-diyl)-4-N-Acetyl-Cytidine (24)

- Et₃N (6.9 mL, 50 mmol) was added to a solution of POCl₃ (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C.
- 5 A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine 23 ([described in example 14] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The
- 10 organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat.
- 15 NaHCO₃ (5mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9
- 20 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 74: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-
ribofuranosyl)-4-N-Acetyl-Cytosine (25)

- 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine 24 (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was
- 25 treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in
- 30 pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine
- 35 (50 mL). The organic extracts were dried over MgSO₄, concentrated *in*

vacuo and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 25 (1.2 g, 1.9 mmol, 68%).

Example 75: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetylcytosine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (26)

5
10
15
1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetylcytosine 25 (0.6 g, 0.97 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product 26, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂:MeOH / 20:1).

Example 76: 2'-Keto-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (28)

20
25
Acetic anhydride (4.6 mL) was added to a solution of 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (Brown, J.; Christodolou, C.; Jones, S.; Modak, A.; Reese, C.; Sibanda, S.; Ubasawa A. *J. Chem. Soc. Perkin Trans. I* 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (4.8 g, 7.2 mmol, 78%).

Example 77: 2'-Deoxy-2'-methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

30
Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g, 17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine

- 28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH₂Cl₂ (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl.
- 5 The organic layer was washed with H₂O (20 mL), 5% aqueous NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 29 (3.86 g, 5.8 mmol, 79%).
- 10

Example 78: 2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

- 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL)
- 15 was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH₂Cl₂.

20 Example 79: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

- 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m.
- 25 The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield 29 (0.81 g, 1.1 mmol, 62%).
- 30

Example 80: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-6-N-(4-*t*-butylbenzoyl)-adenine 29 dissolved in dry CH₂Cl₂ (15 mL) was placed

in a round bottom flask under Ar. Diisopropylethylamine was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76 mmol, 68%). *R*_f 0.45 (CH₂Cl₂: MeOH / 20:1)

10 Example 81: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 82: 2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

25 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-N-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted with 20% MeOH in CH₂Cl₂.

30 Example 83: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-N-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in

pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41 mmol, 69%).

Example 84: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite)

10 (32)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuransyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). 32 (2.3 g, 2.4 mmol, 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.52 (CH₂Cl₂: MeOH / 15:1).

Example 85: 2'-Deoxy-2'-Methoxycarbonylmethylidene-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine (33)

Methyl(triphenylphosphoranylidene)acetate (5.4 g, 16 mmol) was added to a solution of 2'-keto-3',5'-*O*-(tetraisopropyl disiloxane-1,3-diyl)-uridine 14 in CH₂Cl₂ under argon. The mixture was left to stir at RT for 30 h. CH₂Cl₂ (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aq. NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-uridine 33 (5.8 g, 10.8 mmol, 67.5%).

Example 86: 2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (34)

Et₃N·3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5 g, 9.3 mmol) dissolved in CH₂Cl₂ (20 mL) and Et₃N (15 mL). The resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidene-uridine **34** (2.4 g, 8 mmol, 86%) with THF:CH₂Cl₂ / 4:1.

Example 87: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (35)

2'-Deoxy-2'-methoxycarbonylmethylidene-uridine **34** (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH₂Cl₂ as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine **35** (2.03 g, 3.46 mmol, 86%).

Example 88: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (36)

1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine **35** (2.0 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) **36** (1.8 g, 2.3 mmol, 67%) was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.44 (CH₂Cl₂:MeOH / 9.5:0.5).

Example 89: 2'-Deoxy-2'-Carboxymethylidene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine 37

2'-Deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5.0 g, 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **37** (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH₂Cl₂.

The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* PCT WO 94/02595.

Oligonucleotides with 3' and/or 5' Dihalophosphonate

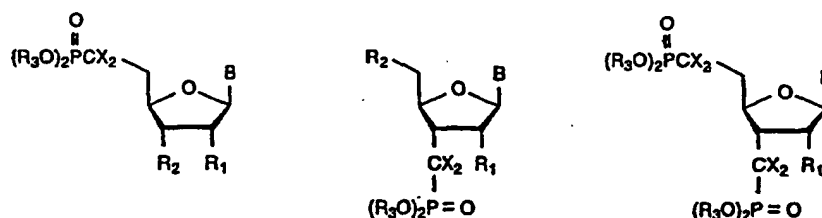
This invention synthesis and uses 3' and/or 5' dihalophosphonate-, e.g., 3' or 5'-CF₂-phosphonate-, substituted nucleotides that maintain or enhance the catalytic activity and/or nuclease resistance of an enzymatic or antisense molecule.

As the term is used in this application, 5'- and/or 3'-dihalophosphonate nucleotide containing ribozymes, deoxyribozymes (see Usman *et al.*, PCT/US94/11649, incorporated by reference herein), and chimeras of nucleotides, are catalytic nucleic molecules that contain 5'-and/or 3'-dihalophosphonate nucleotide components replacing, but not limited to, double-stranded stems, single-stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA or DNA transcript. This invention concerns

nucleic acids formed of standard nucleotides or modified nucleotides, which also contain at least one 5'-dihalophosphonate and/or one 3'-dihalophosphonate group.

The synthesis of 1-O-Ac-2,3-di-O-Bz-D-ribofuranose 5-d-
 5 5-dihalomethylphosphonate in three steps from 1-O-methyl-2,3-O-
 isopropylidene-β-D-ribofuranose 5-deoxy-5-dihalomethylphosphonate is
 described (e.g., for the difluoro, in Figure 87). Condensation of this suitably
 derivatized sugar with silylated pyrimidines and purines affords novel
 nucleoside 5'-deoxy-5'-dihalomethylphosphonates. These intermediates
 10 may be incorporated into catalytic or antisense nucleic acids by either
 chemical (conversion of the nucleoside 5'-deoxy-5'-
 dihalomethylphosphonates into suitably protected phosphoramidites 12a
 or solid supports 12b, e.g., Figure 88) or enzymatic means (conversion of
 the nucleoside 5'-deoxy-5'-dihalomethylphosphonates into their
 15 triphosphates, e.g., 14 Figure 89, for T7 transcription).

Thus, in one aspect the invention features 5' and/or 3'-
 dihalonucleotides and nucleic acids containing such 5' and/or 3'-
 dihalonucleotides. The general structure of such molecules is shown
 below.



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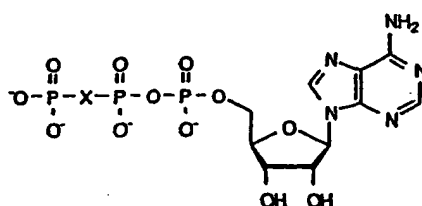
where R₁ is H, OH, or R, where R is a hydroxyl protecting group, e.g.,
 acyl, alkylsilyl, or carbonate; each R₂ is separately H, OH, or R; each R₃ is
 separately a phosphate protecting group, e.g., methyl, ethyl, cyanoethyl, p-
 25 nitrophenyl, or chlorophenyl; each X is separately any halogen; and each B
 is any nucleotide base.

The invention in particular features nucleic acid molecules having
 such modified nucleotides and enzymatic activity. In a related aspect the
 invention features a method for synthesis of such nucleoside 5'-deoxy-5'-
 30 dihalo and/or 3'-deoxy-3'-dihalophosphonates by condensing a

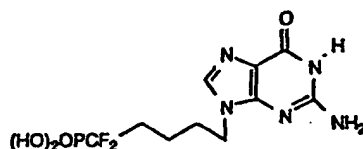
dihalophosphonate-containing sugar with a pyrimidine or a purine under conditions suitable to form a nucleoside 5'-deoxy-5'-dihalophosphonate and/or a 3'-deoxy-3'-dihalophosphonate.

- Phosphonic acids may exhibit important biological properties
- 5 because of their similarity to phosphates (Engel, *Chem. Rev.* 1977, 77, 349-367). Blackburn and Kent (*J. Chem. Soc., Perkin Trans.* 1986, 913-917) indicate that based on electronic and steric considerations *trans*-fluoro and *trans*-difluoromethylphosphonates might mimic phosphate esters better than the corresponding phosphonates. Analogues of pyro- and
- 10 triphosphates 1, where the bridging oxygen atoms are replaced by a difluoromethylene group, have been employed as substrates in enzymatic processes (Blackburn *et al.*, *Nucleosides & Nucleotides* 1985, 4, 165-167; Blackburn *et al.*, *Chem. Scr.* 1986, 26, 21-24). 9-(5,5-Difluoro-5-phosphonopentyl)guanine (2) has been utilized as a multisubstrate
- 15 analogue inhibitor of purine nucleoside phosphorylase (Halazy *et al.*, *J. Am. Chem. Soc.* 1991, 113, 315-317). Oligonucleotides containing methylene groups in place of phosphodiester 5'-oxygens are resistant toward nucleases that cleave phosphodiester linkages between phosphorus and the 5'-oxygen (Breaker *et al.*, *Biochemistry* 1993, 32,
- 20 9125-9128), but can still form stable complexes with complementary sequences. Heinemann *et al.* (*Nucleic Acids Res.* 1991, 19, 427-433) found that a single 3'-methylenephosphonate linkage had a minor influence on the conformation of a DNA octamer double helix.

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3

- One common synthetic approach to α,α -difluoro-alkylphosphonates features the displacement of a leaving group from a suitable reactive substrate by diethyl (lithiodifluoromethyl)phosphonate (3) (Obayashi *et al.*, *Tetrahedron Lett.* 1982, 23, 2323-2326). However, our attempts to synthesize nucleoside 5'-deoxy-5'-difluoro-methylphosphonates from 5'-deoxy-5'-iodonucleosides using 3 were unsuccessful, *i.e.* starting compounds were quantitatively recovered. The reaction of nucleoside 5'-aldehydes with 3, according to the procedure of Martin *et al.* (Martin *et al.*, *Tetrahedron Lett.* 1992, 33, 1839-1842), led to a complex mixture of products. Recently, the synthesis of sugar α,α -difluoroalkylphosphonates from primary sugar triflates using 3 was described (Berkowitz *et al.*, *J. Org. Chem.* 1993, 58, 6174-6176). Unfortunately, our experience is that nucleoside 5'-triflates are too unstable to be used in these syntheses.
- The following are non-limiting examples showing the synthesis of nucleoside 5'-deoxy-5'-difluoromethyl-phosphonates. Those in the art will recognize that equivalent methods can be readily devised based upon

these examples. These examples demonstrate that it is possible to achieve synthesis of 5'-deoxy-5'-difluoro derivatives in good yield and thus guide those in the art to such equivalent methods. The examples also indicate utility of such synthesis to provide useful oligonucleotides as described above.

Those in the art will recognize that useful modified enzymatic nucleic acids can now be designed, much as described by Draper et al., PCT/US94/13129 hereby incorporated by reference herein (including drawings).

10 Example 90: Synthesis of Nucleoside 5'-Deoxy-5'-difluoromethylphosphonates

Referring to Fig. 87, we synthesized a suitable glycosylating agent from the known D-ribose α,α -difluoromethylphosphonate (4) (Martin et al., *Tetrahedron Lett.* 1992, 33, 1839-1842) which served as a key intermediate for the synthesis of nucleoside 5'-difluoromethylphosphonates.

Methyl 2,3-O-isopropylidene- β -D-ribofuranose α,α -difluoromethylphosphonate (4) was synthesized from the 5-aldehyde according to the procedure of Martin et al. (*Tetrahedron Lett.* 1992, 33, 1839-1842) (Figure 87). Removal of the isopropylidene group was accomplished under mild conditions (I_2 -MeOH, reflux, 18 h (Szarek et al., *Tetrahedron Lett.* 1986, 27, 3827) or Dowex 50 WX8 (H^+), MeOH, RT (about 20-25°C), 3 days) in 72% yield. The anomeric mixture thus obtained was benzoylated with benzoyl chloride/pyridine to afford the 2,3-di-O-benzoyl derivative, which was subjected to mild acetolysis conditions (Walczak et al., *Synthesis*, 1993, 790-792) (Ac_2O , AcOH, H_2SO_4 , EtOAc, 0°C. The desired 1-O-acetyl-2,3-di-O-benzoyl-D-ribofuranose difluoromethylphosphonate (5) was obtained in quantitative yield as an anomeric mixture. These derivatives were used for selective glycosylation of silylated uracil and N^4 -acetylcytosine under Vorbrüggen conditions (Vorbrüggen, *Nucleoside Analogs. Chemistry, Biology and Medical Applications*, NATO ASI Series A, 26, Plenum Press, New York, London, 1980; pp. 35-69. The use of $F_3CSO_2OSi(CH_3)_3$ as a glycosylation catalyst is precluded because it is expected to lead to the undesired 1-ethyluracil or 9-ethyladenine byproducts: Podyukova, et al., *Tetrahedron*

Lett. 1987, 28, 3623-3626 and references cited therein) (SnCl_4 as a catalyst, boiling acetonitrile) to yield β -nucleosides (62% 6a, 75% 6b). Glycosylation of silylated N^6 -benzoyladenine under the same conditions yielded a mixture of N-9 isomer 6c and N-7 isomer 7 in 34% and 15% yield, respectively. The above nucleotides were successfully deprotected using trimethylsilylbromide for the cleavage of the ethyl groups, followed by treatment with ammonia-methanol to remove the acyl protecting groups. Nucleoside 5'-deoxy-5'-difluoromethylphosphonates 8 were finally purified on a DEAE Sephadex A-25 (HCO_3^-) column using a 0.01-0.25 M TEAB gradient for elution and obtained as their sodium salts (82% 8a; 87% 8b; 82% 8c).

Selected analytical data: ^{31}P -NMR (^{31}P) and ^1H -NMR (^1H) were recorded on a Varian Gemini 400. Chemical shifts in ppm refer to H_3PO_4 and TMS, respectively. Solvent was CDCl_3 unless otherwise noted. 5: ^1H δ 8.07-7.28 (m, Bz), 6.66 (d, $J_{1,2}$ 4.5, $\alpha\text{H}1$), 6.42 (s, $\beta\text{H}1$), 5.74 (d, $J_{2,3}$ 4.9, $\beta\text{H}2$), 5.67 (dd, $J_{3,2}$ 4.9, $J_{3,4}$ 6.6, $\beta\text{H}3$), 5.63 (dd, $J_{3,2}$ 6.7, $J_{3,4}$ 3.6, $\alpha\text{H}3$), 5.57 (dd, $J_{2,1}$ 4.5, $J_{2,3}$ 6.7, $\alpha\text{H}2$), 4.91 (m, H4), 4.30 (m, CH_2CH_3), 2.64 (m, CH_2CF_2), 2.18 (s, βAc), 2.12 (s, αAc), 1.39 (m, CH_2CH_3). ^{31}P δ 7.82 (t, $J_{\text{P,F}}$ 105.2), 7.67 (t, $J_{\text{P,F}}$ 106.5). 6a: ^1H δ 9.11 (s, 1H, NH), 8.01 (m, 11H, Bz, H6), 5.94 (d, $J_{1',2'}$ 4.1, 1H, H1'), 5.83 (dd, $J_{5,6}$ 8.1, 1H, H5), 5.79 (dd, $J_{2',1'}$ 4.1, $J_{2',3'}$ 6.5, 1H, H2'), 5.71 (dd, $J_{3',2'}$ 6.5, $J_{3',4'}$ 6.4, 1H, H3'), 4.79 (dd, $J_{4',3'}$ 6.4, $J_{4',\text{F}}$ 11.6, 1H, H4'), 4.31 (m, 4H, CH_2CH_3), 2.75 (tq, $J_{\text{H,F}}$ 19.6, 2H, CH_2CF_2), 1.40 (m, 6H, CH_2CH_3). ^{31}P δ 7.77 (t, $J_{\text{P,F}}$ 104.0). 8c: ^{31}P (vs DSS) (D_2O) δ 5.71 (t, $J_{\text{P,F}}$ 87.9).

Compound 7 was deacylated with methanolic ammonia yielding the product that showed λ_{max} (H_2O) 271 nm and λ_{min} 233 nm, confirming that the site of glycosylation was N-7.

Example 91: Synthesis of Nucleic Acids Containing Modified Nucleotide Containing Cores

The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman *et al.*, *J. Am. Chem. Soc.* 1987, 109, 7845-7854 and in Scaringe *et al.*, *Nucleic Acids Res.* 1990, 18, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (Figure 88 and Janda *et al.*, *Science* 1989, 244:437-440.). These

nucleoside 5'-deoxy-5'-difluoromethylphosphonates may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group 1 or Group 2 introns, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

5 Example 92: Synthesis of Modified Triphosphate

The triphosphate derivatives of the above nucleotides can be formed as shown in Fig. 89, according to known procedures. *Nucleic Acid Chem.*, Leroy B. Townsend, John Wiley & Sons, New York 1991, pp. 337-340; *Nucleotide Analogs*, Karl Heinz Scheit; John Wiley & Sons New York 1980, pp. 211-218.

Equivalent synthetic schemes for 3' dihalophosphonates are shown in Figures 90 and 91 using art recognized nomenclature. The conditions can be optimized by standard procedures.

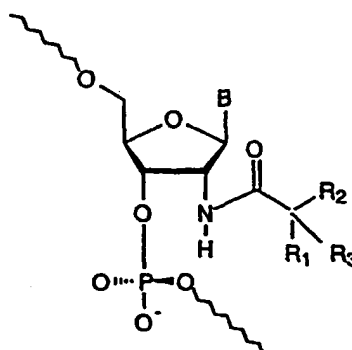
The nucleoside dihalophosphonates described herein are advantageous as modified nucleotides in any nucleic acid structure, e.g., catalytic or antisense, since they are resistant to exo- and endonucleases that normally degrade unmodified nucleic acids *in vivo*. They also do not perturb the normal structure of the nucleic acid in which they are incorporated thereby maintaining any activity associated with that structure.

20 These compounds may also be of use as monomers as antiviral and/or antitumor drugs.

Oligonucleotides with Amido or Peptido Modification

This invention replaces 2'-hydroxyl group of a ribonucleotide moiety with a 2'-amido or 2'-peptido moiety. In other embodiments, the 3' and 5' portions of the sugar of a nucleotide may be substituted, or the phosphate group may be substituted with amido or peptido moieties. Generally, such a nucleotide has the general structure shown in Formula I below:

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FORMULA I

The base (B) is any one of the standard bases or is a modified nucleotide base known to those in the art, or can be a hydrogen group. In addition, either R_1 or R_2 is H or an alkyl, alkene or alkyne group containing between 2 and 10 carbon atoms, or hydrogen, an amine (primary, secondary or tertiary, *e.g.*, R_3NR_4 where each R_3 and R_4 independently is hydrogen or an alkyl, alkene or alkyne having between 2 and 10 carbon atoms, or is a residue of an amino acid, *i.e.*, an amide), an alkyl group, or an amino acid (D or L forms) or peptide containing between 2 and 5 amino acids. The zigzag lines represent hydrogen, or a bond to another base or other chemical moiety known in the art. Preferably, one of R_1 , R_2 and R_3 is an H, and the other is an amino acid or peptide.

Applicant has recognized that RNA can assume a much more complex structural form than DNA because of the presence of the 2'-hydroxyl group in RNA. This group is able to provide additional hydrogen bonding with other hydrogen donors, acceptors and metal ions within the RNA molecule. Applicant now provides molecules which have a modified amine group at the 2' position, such that significantly more complex structures can be formed by the modified oligonucleotide. Such modification with a 2'-amido or peptido group leads to expansion and enrichment of the side-chain hydrogen bonding network. The amide and peptide moieties are responsible for complex structural formation of the oligonucleotide and can form strong complexes with other bases, and interfere with standard base pairing interactions. Such interference will allow the formation of a complex nucleic acid and protein conglomerate.

Oligonucleotides of this invention are significantly more stable than existing oligonucleotides and can potentially form biologically active bioconjugates not previously possible for oligonucleotides. They may also be used for *in vitro* selection of unique aptamers, that is, randomly generated oligonucleotides which can be folded into an effective ligand for a target protein, nucleic acid or polysaccharide.

Thus, in one aspect, the invention features an oligonucleotide containing the modified base shown in Formula I, above.

In other aspects, the oligonucleotide may include a 3' or 5' nucleotide having a 3' or 5' located amino acid or aminoacyl group. In all these aspects, as well as the 2'-modified nucleotide, it will be evident that various standard modifications can be made. For example, an "O" may be replaced with an S, the sugar may lack a base (i.e., abasic) and the phosphate moiety may be modified to include other substitutions (see Sproat, *supra*).

Example 93: General procedure for the preparation of 2'-aminoacyl-2'-deoxy-2'-aminonucleoside conjugates.

Referring to Fig. 92, to the solution of 2'-deoxy-2'-amino nucleoside (1 mmol) and N-Fmoc L- (or D-) amino acid (1 mmol) in methanol [dimethylformamide (DMF) and tetrahydrofuran (THF) can also be used], 1-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) [or 1-isobutyloxycarbonyl-2-isobutyloxy-1,2-dihydroquinoline (IIDQ)] (2 mmol) is added and the reaction mixture is stirred at room temperature or up to 50 °C from 3-48 hours. Solvents are removed under reduced pressure and the residual syrup is chromatographed on the column of silica-gel using 1-10 % methanol in dichloromethane. Fractions containing the product are concentrated yielding a white foam with yields ranging from 85 to 95 %. Structures are confirmed by ¹H NMR spectra of conjugates which show correct chemical shifts for nucleoside and aminoacyl part of the molecule. Further proofs of the structures are obtained by cleaving the aminoacyl protecting groups under appropriate conditions and assigning ¹H NMR resonances for the fully deprotected conjugate.

Partially protected conjugates described above are converted into their 5'-O-dimethoxytrityl derivatives and into 3'-phosphoramidites using standard procedures (Oligonucleotide Synthesis: A Practical Approach,

M.J. Gait ed.; IRL Press, Oxford, 1984). Incorporation of these phosphoramidites into RNA was performed using standard protocols (Usman *et al.*, 1987 *supra*).

5 A general deprotection protocol for oligonucleotides of the present invention is described in Fig. 93.

The scheme shows synthesis of conjugate of 2'-d-2'-aminouridine. This is meant to be a non-limiting example, and those skilled in the art will recognize that, variations to the synthesis protocol can be readily generated to synthesize other nucleotides (e.g., adenosine, cytidine,
10 guanosine) and/or abasic moieties.

Example 94: RNA cleavage by hammerhead ribozymes containing 2'-aminoacyl modifications.

Hammerhead ribozymes targeted to site N (see Fig. 94) are synthesized using solid-phase synthesis, as described above. U4 and U7
15 positions are modified, individually or in combination, with either 2'-NH-alanine or 2'-NH-lysine.

RNA cleavage assay *in vitro*: Substrate RNA is 5' end-labeled using [γ -³²P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace
20 amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is initiated by mixing the
25 ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of
30 time.

Referring to Fig. 95, hammerhead ribozymes containing 2'-NH-alanine or 2'-NH-lysine modifications at U4 and U7 positions cleave the target RNA efficiently.

Sequences listed in Figure 94 and the modifications described in Figure 95 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 95: Aminoacylation of 3'-ends of RNA

I. Referring to Fig. 96, 3'-OH group of the nucleotide is converted to succinate as described by Gait, *supra*. This can be linked with amino-alkyl solid support (for example: CpG). Zig-zag line indicates linkage of 3'OH group with the solid support.

II. Preparation of aminoacyl-derivatized solid support

A) Synthesis of O-Dimethoxytrityl (O-DMT) amino acids

Referring to Fig. 97, to a solution of L- (or D-) serine, tyrosine or threonine (2 mmol) in dry pyridine (15 ml) 4,4'-dimethoxytrityl chloride (3 mmol) is added and the reaction mixture is stirred at RT (about 20-25°C) for 16 h. Methanol (10 ml) is then added and the solution evaporated under reduced pressure. The residual syrup was partitioned between 5% aq. NaHCO₃ and dichloromethane, organic layer was washed with brine, dried (Na₂SO₄) and concentrated *in vacuo*. The residue is purified by flash silicagel column chromatography using 2-10% methanol in dichloromethane (containing 0.5 % pyridine). Fractions containing product are combined and concentrated *in vacuo* to yield white foam (75-85 % yield).

B) Preparation of the solid support and its derivatization with amino acids

Referring to Fig. 97, the modified solid support (has an OH group instead of the standard NH₂ end group) was prepared according to Haralambidis et al., *Tetrahedron Lett.* 1987, 28, 5199, (P denotes aminopropyl CPG or polystyrene type support). O-DMT or NH-monomethoxytrityl (NH-MMT amino acid was attached to the above solid support using standard procedures for derivatization of the solid support (Gait, 1984, *supra*) creating a base-labile ester bond between amino acids

and the support. This support is suitable for the construction of RNA/DNA chain using suitably protected nucleoside phosphoramidites.

Example 96: Aminoacylation of 5'-ends of RNA

- I. Referring to Fig. 98, 5'-amino-containing sugar moiety was synthesized as described (Mag and Engels, 1989 *Nucleic Acids Res.* 17, 5973). Aminoacylation of the 5'-end of the monomer was achieved as described above and RNA phosphoramidite of the 5'-aminoacylated monomer was prepared as described by Usman *et al.*, 1987 *supra*. The phosphoramidite was then incorporated at the 5'-end of the oligonucleotide using standard solid-phase synthesis protocols described above.
- II. Referring to Fig. 99, aminoacyl group(s) is attached to the phosphate group at the 5'-end of the RNA using standard procedures described above.

VII. Reversing Genetic Mutations

- Modification of existing nucleic acid sequences can be achieved by homologous recombination. In this process a transfected sequence recombines with homologous chromosomal sequences and can replace the endogenous cellular sequence. Boggs, 8 *International J. Cell Cloning* 80, 1990, describes targeted gene modification. It reviews the use of homologous DNA recombination to correct genetic defects. Banga and Boyd, 89 *Proc. Natl. Acad. Sci. U.S.A.* 1735, 1992, describe a specific example of *in vivo* site-directed mutagenesis using a 50 base oligonucleotide. In this methodology a gene or gene segment is essentially replaced by the oligonucleotide used.
- This invention uses a complementary oligonucleotide to position a nucleotide base changing activity at a particular site on a gene (RNA or genomic DNA), such that the nucleotide modifying activity will change (or revert) a mutation to wild-type, or its equivalent. By reversion or change of a mutation, we refer to reversion in a broad sense, such as when a mutation at a second site which leads to functional reversion to a wild type phenotype. Also, due to the degeneracy of the genetic code, a revertant may be achieved by changing any one of the three codon positions. Additionally, creation of a stop codon in a deleterious gene (or transcript) is defined here as reverting a mutant phenotype to wild-type. An example of

this type of reversion is creating a stop codon in a critical HIV proviral gene in a human.

Referring to Figures 100 and 101, broadly there are two approaches to causing a site directed change in order to revert a mutation to wild-type.

5 In one (Fig. 100) the oligonucleotide is used to target RNA specifically. RNA is provided with a complementary (Watson-crick) oligonucleotide sequence to that in the target molecule. In this case the sequence modifying oligonucleotide would (analogously to an antisense oligonucleotide or ribozyme) have to be continuously present to revert the
10 RNA as it is made by the cell. Such a reversion would be transient and would potentially require continuous addition of more sequence modifying oligonucleotide. The transient nature of this approach is an advantage, in that treatment could be stopped by simply removing the sequence modifying oligonucleotide (as with a traditional drug).

15 A second approach targets DNA (Fig. 101) and has the advantage that changes may be permanently encoded in the target cell's genetic code. Thus, a single course (or several courses) of treatment may lead to permanent reversion of the genetic disease. If inadvertent chromosomal mutations are introduced this may cause cancer, mutate other genes, or
20 cause genetic changes in the germ-line (in patients of reproductive age). However, if the base changing activity is a specific methylation that may modulate gene expression it would not necessarily lead to germ-line transmission. See Lewin, Genes, 1983 John Wiley & Sons, Inc. NY pp 493-496.

25 Complementary base pairing to single-stranded DNA or RNA is one method of directing an oligonucleotide to a particular site of DNA. This could occur by a strand displacement mechanism or by targeting DNA when it is single-stranded (such as during replication, or transcription). Another method is using triple-strand binding (triplex formation) to double-
30 stranded DNA, which is an established technique for binding polypyrimidine tracts, and can be extended to recognize all 4 nucleotides. See Povsic, T., Strobel, S., & Dervan, P. (1992). Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation. J. Am. Chem. Soc. 114, 5934-5944 (1992). Knorre, D.G., Valentin, V.V.,
35 Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk,

1993) describe conjugation of reactive groups or enzyme to oligonucleotides and can be used in the methods described herein.

Recently, antisense oligonucleotides have been used to redirect an incorrect splice into order to obtain correct splicing of a splice mutant globin gene *in vitro*. Dominski Z; Kole R (1993) Restoration of correct splicing in thalassemia pre-mRNA by antisense oligonucleotides. Proc Natl Acad Sci U S A 90:8673-7. Analogously, in one preferred embodiment of this invention a complementary oligomer is used to correct an existing mutant RNA, instead of the traditional approach of inhibiting that RNA by antisense.

In either the RNA or DNA mode, after binding to a particular site on the RNA or DNA the oligonucleotide will modify the nucleic acid sequence. This can be accomplished by activating an endogenous enzyme (see Figure 102), by appropriate positioning of an enzyme (or ribozyme) conjugated (or activated by the duplex) to the oligonucleotide, or by appropriate positioning of a chemical mutagen. Specific mutagens, such as nitrous acid which deaminates C to U, are most useful, but others can also be used if inactivation of a harmful RNA is desired.

RNA editing is an naturally occurring event in mammalian cells in which a sequence modifying activity edits a RNA to its proper sequence post-transcriptionally. Higuchi, M., Single, F., Kohler, M., Sommer, B., and Seeburg, P. (1993) RNA Editing of AMPA Receptor Subunit GluR-B: A base-paired intron-exon structure determines position and efficiency Cell 75:1361-1370. The machinery involved in RNA editing can be co-opted by a suitable oligonucleotide in order to promote chemical modification.

The changes in the base created by the methods of this invention cause a change in the nucleotide sequence, either directly, or after DNA repair by normal cellular mechanisms. These changes functionally correct a genetic defect or introduce a stop codon. Thus, the invention is distinct from techniques in which an active chemical group (e.g., an alkylator) is attached to an antisense or triple strand oligonucleotide in order to chemically inactivate the target RNA or DNA.

Thus, this invention creates an alteration to an existing base in a nucleic acid molecule so that the base is read *in vivo* as a different base.

This includes correcting a sequence instead of inactivating a gene but can also include inactivating a deleterious gene.

Thus, in one aspect, the invention features a method for altering *in vivo* the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule. The method includes contacting the nucleic acid molecule *in vivo* with an oligonucleotide or peptide nucleic acid or other sequence specific binding molecules able to form a duplex or triplex molecule with the nucleic acid molecule. After formation of the duplex or triplex molecule a base modifying activity chemically or enzymatically alters the targeted base directly, or after nucleic acid repair *in vivo*. This results in the functional alteration of the nucleic acid sequence.

By "alter", as it is used in this context, is meant that one or more chemical moieties in a targeted base, or bases, is altered so that the mutant nucleic acid will be functionally different. Thus, this is distinct from prior methods of correcting defects in DNA, such as homologous recombination, in which an entire segment of the targeted sequence is replaced with a segment of DNA from the transfected nucleic acid. This is also distinct from other methods that use reactive groups to inactivate a RNA or DNA target, in that this method functionally corrects the sequence of the target, instead of merely damaging it, by causing it to be read by a polymerase as a different base from the original base. As noted above, the naturally occurring enzymes in a cell can be utilized to cause the chemical alteration, examples of which are provided below.

By "functionally alter" is meant that the ability of the target nucleic acid to perform its normal function (*i.e.*, transcription or translation control) is changed. For example, an RNA molecule may be altered so that it can cause production of a desired protein, or a DNA molecule can be altered so that upon DNA repair, the DNA sequence is changed.

By "mutant" it is meant a nucleic acid molecule which is altered in some way compared to equivalent molecules present in a normal individual. Such mutants may be well known in the art, and include, molecules present in individuals with known genetic deficiencies, such as muscular dystrophy, or diabetes and the like. It also includes individuals with diseases or conditions characterized by abnormal expression of a gene, such as cancer, thalassemia's and sickle cell anemia, and cystic

fibrosis. It allows modulation of lipid metabolism to reduce artery disease, treatment of integrated AIDS genomes, and AIDS RNA, and Alzheimer's disease. Thus, this invention concerns alteration of a base in a mutant to provide a "wild type" phenotype and/or genotype. For deleterious conditions this involves altering a base to allow expression or prevent expression as is necessary. When treating an infection, such as HIV, it concerns inactivation of a gene in the HIV RNA by mutation of the mutant (*i.e.*, non-human gene) to a wild type (*i.e.*, no production of a non-human protein). Such modification is performed *in trans* rather than *in cis* as in prior methods.

In preferred embodiments, the oligonucleotide is of a length (at least 12 bases, preferably 17 - 22) sufficient to activate dsRNA deaminase *in vivo* to cause conversion of an adenine base to inosine; the oligonucleotide is an enzymatic nucleic acid molecule that is active to chemically modify a base (see below); the nucleic acid molecule is DNA or RNA; the oligonucleotide includes a chemical mutagen, *e.g.*, the mutagen is nitrous acid; and the oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.

In a most preferred embodiment, the invention features correction of a mutation, rather than inactivation of a target by causing a mutation.

Using *in vitro* directed evolution, it is possible to screen for ribozymes with catalytic activities different than RNA cleavage. Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418. Using these methods of *in vitro* directed evolution, an enzymatic nucleic acid molecule, or ribozyme that mutates bases, instead of cleaving the phosphodiester backbone can be selected. This is a convenient method of obtaining an enzyme with the appropriate base sequence modifying activities for use in the present invention.

Sequence modifying activities can change one nucleotide to another (or modify a nucleotide so that it will be repaired by the cellular machinery to another nucleotide). Sequence modifying activities could also delete or add one or more nucleotides to a sequence. A specific embodiment of adding sequences is described by Sullenger and Cech, PCT/US94/12976

hereby incorporated by reference herein), in which entire exons with wild-type sequence are spliced into a mutant transcript. The present invention features only the addition of a few bases (1 - 3).

Thus, in another aspect, the invention features ribozymes or enzymatic nucleic acid molecules active to change the chemical structure of an existing base in a separate nucleic acid molecule. Applicant is the first to determine that such molecules would be useful, and to provide a description of how such molecules might be isolated.

Molecules used to achieve *in situ* reversion can be delivered using the existing means employed for delivering antisense molecules and ribozymes, including liposomes and cationic lipid complexes. If the *in situ* reverting molecule is composed only of RNA, then expression vectors can be used in a gene therapy protocol to produce the reverting molecules endogenously, analogously to antisense or ribozymes expression vectors. There are several advantages of using such an expression vector, rather than simply replacing the gene through standard gene therapy. Firstly, this approach would limit the production of the corrected gene to cells that already express that gene. Furthermore, the corrected gene would be properly regulated by its natural transcriptional promoter. Lastly, reversion can be used when the mutant RNA creates a dominant gain of function protein (e.g., in sickle cell anemia), where correction of the mutant RNA is necessary to stop the production of the deleterious mutant protein, and allow production of the corrected protein.

Endogenous Mammalian RNA Editing System

It was observed in the mid-1980s that the sequence of certain cellular RNAs were different from the DNA sequence that encodes them. By a process called RNA editing, cellular RNA are post-transcriptionally modified to a) create a translation initiation and termination codons, b) enable tRNA and rRNA to fold into a functional conformation (for a review see Bass, B. L. (1993) In The RNA World, R. Gesteland, R. and Atkins, J. eds. (Cold Spring Harbor, New York; CSH Lab. Press) pp. 383-418). The process of RNA editing includes base modification, deletion and insertion of nucleotides.

Although, the RNA editing process is widespread among lower eukaryotes, very few RNAs (four) have been reported to undergo editing in

mammals (Bass, *supra*). The predominant mode of RNA editing in mammalian system is base modification ($C \rightarrow U$ and $A \rightarrow G$). The mechanism of RNA editing in the mammalian system is postulated to be that $C \rightarrow U$ conversion is catalyzed by cytidine deaminase. The mechanism of conversion of $A \rightarrow G$ has recently been reported for glutamate receptor B subunit (gluR-B) in rat PC12 cells (Higuchi, M. et al. (1993) *Cell* 75, 1361-1370). According to Higuchi gluR-B mRNA precursor attains a structure such that intron 11 and exon 11 can form a stable stem-loop structure. This stem-loop structure is a substrate for a nuclear double strand-specific adenosine deaminase enzyme. The deamination will result in the conversion of $A \rightarrow I$. Reverse transcription followed by double strand synthesis will result in the incorporation of G in place of A.

In the present invention, the endogenous deaminase activity or other such activities can be utilized to achieve targeted base modification.

The following are examples of the invention to illustrate different methods by which *in vivo* conversion of a base can be achieved. These are provided only to clarify specific embodiments of the invention and are not limiting to the invention. Those in the art will recognize that equivalent methods can be readily devised within the scope of the claims.

Example 97: Exploiting cellular dsRNA dependent Adenine to Inosine converter;

An endogenous activity in most mammalian cells and *Xenopus* oocytes converts about 50% of adenines to inosines in double stranded RNA. (Bass, B. L., & Weintraub, H. (1988). An unwinding activity that covalently modifies it double-stranded RNA substrate. *Cell*, 55, 1089-1098.). This activity can be used to cause an *in situ* reversion of a mutation at the RNA level. Referring to Figures 102 and 104, for demonstration purposes a stop codon is incorporated into the coding region of dystrophin, which is fused to the reporter gene luciferase. This stop codon can be reverted by targeting an antisense RNA which is long enough to activate the dsRNA deaminase, which converts Adenines to Inosines. The A to I transition will be read by the ribosome as an A to G transition in some cases and will thereby functionally revert the stop codon. While other A's in this region may be converted to I's and read as G, converting an A to I (G) cannot create a stop codon. The A to I transitions

in the region surrounding the target mutation will create some point mutations, however, the function of the dystrophin protein is rarely inactivated by point mutations.

The reverted mRNA was then translated in a cell lysate and assayed for luciferase activity. As evidenced by the dramatic increase in luciferase counts in the graph in figure 103, the A to I transition was read by the ribosome as an A to G transition and the stop codon has successfully been reverted with the lysate treated complex. As a control, an irrelevant non-complementary RNA oligonucleotide was added to the dystrophin/luciferase mRNA. As expected, in this case no translation (luciferase activity) is observed because of the stop codon. As an additional control, the hybrid was not treated with extract, and again no translation (luciferase activity) is observed (Figure 103).

While other A's in the targeted region may have been converted to I's and read as G, converting an A to I (G) cannot create a stop codon, so the ribosome will still read through the region. Dystrophin is not generally sensitive to point mutations if the open reading frame is maintained, so a dystrophin protein made from an mRNA reverted by this method should retain full activity.

The following detail specifics of the methodology: RNA oligonucleotides were synthesized on a 394 (ABI) synthesizer using phosphoramidite chemistry. The sequence of the synthetic complementary RNA that binds to the mutant dystrophin sequence is as follows (5' to 3'):

CCCGCGGTAGATCTTTCTGGAGGCTTACAGTTTCTACAAACCTCC
CTTCAAA (Seq. ID No. 1)

Referring to Figure 104, fifty-nine base pairs of a human dystrophin mutant sequence containing a stop codon was fused in frame to the luciferase coding region using standard cloning technology, into the *Hind* III and *Not* I sites of pRC-CMV (Invitrogen, San Diego, CA). The AUG of luciferase was deleted. The sequences of the insert from the *Hind* III site to the start of the luciferase coding region is (5' to 3'):

GCCCCTGAGGAGCGATGGAGGCCTTGAAGGGAGGTTTGTGAAAA
CTGTAAGCCTCCAGAAAGATCTACCGCGG (Seq ID No. 2)

This corresponds to base pairs 3649-3708 of normal dystrophin (Entrez ID # 311627) with a *Sac* II site at the 3' end. This plasmid was used as a template for *in vitro* transcription of mRNA using T7 polymerase with the manufacturers protocol (Promega, Madison, WI).

5 *Xenopus* nuclear extracts were prepared in 0.5X TGKED buffer (0.5X= 25mM Tris (pH 7.9), 12.5% glycerol, 25 mM KCl, 0.25mM DTT and 0.05mM EDTA), by vortexing nuclei and resuspended in a volume of 0.5X TGKED equal to total cytoplasm volume of the oocytes. Bass, B.L. & Weintraub, H. *Cell* 55, 1089-1098 (1988).

10 The target mRNA at 500ng/ul was pre-annealed to 1 micromolar complementary or irrelevant RNA oligonucleotide by heating to 70°C, and allowing it to slowly cool to 37°C over 30 minutes. Fifty nanograms of mRNA pre-annealed to the RNA oligonucleotides was added to 7ul of nuclear extracts containing 1mM ATP, 15mM EDTA, 1600un/ml RNasin
15 and 12.5mM Tris pH 8 to a total volume of 12ul. Bass, B.L. & Weintraub, H. *supra*. This mixture, which contains the dsRNA deaminase activity, was incubated for 30 minutes at 25°C. Next, 1.5ul of this mixture was added to a rabbit reticulocyte lysate *in vitro* translation mixture and translated for two hours according to the manufacturers protocol (Life Technologies,
20 Gaithersburg, MD), except that an additional 1.3 mM magnesium acetate was added to compensate for the EDTA carried through from the nuclear extract mixture. Luciferase assays were performed on 15ul of extract with the Promega luciferase assay system (Promega, Madison, WI), and luminescence was detected with a 96 well luminometer, and the results are
25 displayed in the graph in figure 102.

Example 98: Base changing activities

The chemical synthesis of antisense and triple-strand forming oligomers conjugated to reactive groups is well studied and characterized (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova,
30 O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993) and Povsic, T., Strobel, S. & Dervan, P. Sequence-specific double-strand alkylation and cleavage of DNA mediated by triple-helix formation *J. Am. Chem. Soc.* 114, 5934-5944 (1992). Reactive groups such as alkylators that can modify nucleotide
35 bases in targeted RNA or DNA have been conjugated to oligonucleotides.

Additionally enzymes that modify nucleic acids have been conjugated to oligonucleotides. (Knorre, D.G., Valentin, V.V., Valentina, F.Z., Lebedev, A.V. & Federova, O.S. *Design and targeted reactions of oligonucleotide derivatives* 1-366 (CRC Press, Novosibirsk, 1993). In the past these
5 conjugated chemical groups or enzymes have been used to inactivate DNA or RNA that is specifically targeted by antisense or triple-strand interactions. Below is a list of useful base changing activities that could be used to change the sequence of DNA or RNA targeted by antisense or triple strand interactions, in order to achieve *in situ* reversion of mutations,
10 as described herein (see figure 100-104).

1. Deamination of 5-methylcytosine to create thymidine (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993). Also, nitrous acid or related compounds promote oxidative deamination of
15 C to be read at T (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.). Additionally hydroxylamine or related compounds can transform C to be read at T (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc., Boston, 1987, PP.226-230.)

20 2. Deamination of cytosine to create uracil (performed by the enzyme cytidine deaminase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993) or by chemical groups similar to nitrous acid that promote oxidative deamination (Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Inc.,
25 Boston, 1987, PP.226-230.)

3. Deamination of Adenine to be read like G (Inosine) (as done by the adenosine deaminase, AMP deaminase or the dsRNA deaminating activity (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).

30 4. Methylation of cytosine to 5-methylcytosine

5. Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine by alkynitrosoureas (Xu, and Swann, *Tetrahedron Letters* 35:303-306 (1994)).

6. Transforming guanine to 6-O-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, *Biochimica et Biophysica Acta*, 521:770-778 (1978) which can be done with the mutagen ethyl methane sulfonate (EMS) Microbial Genetics, David Freifelder, Jones and Bartlett
5 Publishers, Inc., Boston, 1987, PP.226-230.

7. Amination of uracil to cytosine (as performed by the cellular enzyme CTP synthetase (Bass, B.L. in *The RNA World* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, 1993).

- The following are examples of useful chemical modifications that can
10 be utilized in the present invention. There are a few preferred straightforward chemical modifications that can change one base to another base. Appropriate mutagenic chemicals are placed on the targetting oligonucleotide, *e.g.*, nitrous acid, or a suitable protein with such activity. Such chemicals and proteins can be attached by standard
15 procedures. These include molecules which introduce fundamental chemical changes, that would be useful independent of the particular technical approach. See Lewin, *Genes*, 1983 John Wiley & Sons, Inc. NY pp 42-48.

- The following matrix shows that the chemical modifications noted can
20 cause transversion reversions (pyrimidine to pyrimidine, or purine to purine) in RNA or DNA. The transversions (pyrimidine to purine, or purine to pyrimidine) are not preferred because these are more difficult chemical transformations. The footnotes refer to the specific desired chemical transformations. The bold footnotes refer to the reaction on the opposite
25 DNA strand. For example, if one desires to change an A to a G, this can be accomplished at the DNA level by using reaction #5 to change a T to a C in the opposing strand. In this example an A/T base pair goes to A/C, then when the DNA is replicated, or mismatch repair occurs this can become G/C, thus the original A has been converted to a G.

30

ISR matrix

Reverted Base

Mutant base	A	T(U)	C	G
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161

A	-	Transversion	Transversion	DNA ^{5,3} /RNA ³
T(U)	Transversion	-	DNA ⁵ /RNA ⁷	Transversion
C	Transversion	RNA ² /DNA ⁶	-	Transversion
G	DNA ⁶ /RNA ⁶	Transversion	Transversion	-

- 1 Deamination of 5-methylcytosine to create thymidine.
- 2 Deamination of cytosine to create uracil.
- 3 Deamination of Adenine to be read like G (Inosine).
- 5 4 Methylation of cytosine to 5-methylcytosine.
- 5 5 Transforming thymidine (or uracil) to O²-methyl thymidine (or O²-methyl uracil), to be read as cytosine (Xu, and Swann, Tetrahedron Letters 35:303-306 (1994)).
- 10 6 Transforming guanine to 6-O-methyl (or other alkyls) to be read as adenine (Mehta and Ludlum, Biochimica et Biophysica Acta, 521:770-778 (1978)).
7. Amination of uracil to cytosine. Bass *supra*. fig. 6c.

In Vitro Selection Strategy

- Referring to Figure 105, there is provided a schematic describing an approach to selecting for a ribozyme with such base changing activity.
- 15 An RNA is designed that folds back on itself (this is similar to approaches already used to select for RNA ligases, Bartel, D. and Szostak, J. (1993) Isolation of new ribozymes from a large pool of random sequences. Science 261:1411-1418). A degenerate loop opposing the base to be
 - 20 modified provides for diversity. After incubating this library of molecules in a buffer, the RNA is reverse transcribed into DNA (that is, using standard *in vitro* evolution protocol. Tuerk and Gold, 249 Science 505, 1990) , and then the DNA is selected for having a base change. A restriction enzyme cleavage and size selection or its equivalent is used to isolate the fraction
 - 25 of DNAs with the appropriate base change. The cycle could then be repeated many times.

The *in vitro* selection (evolution) strategy is similar to approaches developed by Joyce (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641; Joyce, G. F. (1992) Scientific American 267, 90-97) and Szostak (Bartel, D. and Szostak, J. (1993) Science 261:1411-1418; Szostak, J. W. (1993) TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein, each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (the region flanking the mutant nucleotide), 2) complimentary DNA (cDNA) synthesis and PCR amplification of molecules selected for their base modifying activity, 3) introduction of restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry, A. A. and Joyce, G.F. (1992) Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences (see Figure 105), such that the degenerate domain is placed across from the mutant base (the base that is targeted for modification). This random library of nucleic acids is incubated under conditions that ensure folding of the nucleic acids into conformations that facilitate the catalysis of base modification (the reaction protocol may also include certain cofactors like ATP or GTP or an S-adenosyl-methionine (if methylation is desired) in order to make the selection more stringent). Following incubation, nucleic acids are converted into complimentary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with base modification (at the mutant base position) can be separated from rest of the population of nucleic acids by using a variety of methods. For example, a restriction endonuclease cleavage site can either be created or abolished as a result of base modification. If a restriction endonuclease site is created as a result of base modification, then the library can be digested with the restriction endonuclease (RE). The fraction of the population that is cleaved by the RE is the population that has been able to catalyze the base modification reaction (active pool). A new piece of DNA (containing oligonucleotide primer binding sites for PCR and RE sites for cloning) is ligated to the termini of the active pool to facilitate PCR amplification and subsequent cycles (if necessary) of selection. The final pool of nucleic acids with the best base modifying activity is cloned in to a plasmid vector

and transformed into bacterial hosts. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

Base modifying enzymatic nucleic acids (identified via *in vitro* selection) can be used to cause the chemical modification *in vivo*.

In addition, the ribozyme could be evolved to specifically bind a protein having an enzymatic base changing activity.

Such ribozymes can be used to cause the above chemical modifications *in vivo*. The ribozymes or above noted antisense-type molecules can be administered by methods discussed in the above referenced art.

VIII. Administration of Nucleic Acids

Applicant has determined that double-stranded nucleic acid lacking a transcription termination signal can be used for continuous expression of the encoded RNA. This is achieved by use of an R-loop, *i.e.*, an RNA molecule non-covalently associated with the double-stranded nucleic acid and which causes localized denaturation ("bubble" formation) within the double stranded nucleic acid (Thomas et al., 1976 Proc. Natl. Acad. Sci. USA 73, 2294). In addition, applicant has determined that the RNA portion of the R-loop can be used to target the whole R-loop complex to a desirable intracellular or cellular site, and aid in cellular uptake of the complex. Further, applicant indicates that expression of enzymatically active RNA or ribozymes can be significantly enhanced by use of such R-loop complexes.

Thus, in one aspect, the invention features a method for introduction of enzymatic nucleic acid into a cell or tissue. A complex of a first nucleic acid encoding the enzymatic nucleic acid and a second nucleic acid molecule is provided. The second nucleic acid molecule has sufficient complementarity with the first nucleic acid to be able to form an R-loop base pair structure under physiological conditions. The R-loop is formed in a region of the first nucleic acid molecule which promotes expression of RNA from the first nucleic acid under physiological conditions. The method further includes contacting the complex with a cell or tissue under

conditions in which the enzymatic nucleic acid is produced within the cell or tissue.

By "complex" is simply meant that the two nucleic acid molecules interact by intermolecular bond formation (such as by hydrogen bonding) between two complementary base-paired sequences. The complex will generally be stable under physiological condition such that it is able to cause initiation of transcription from the first nucleic acid molecule.

The first and second nucleic acid molecules may be formed from any desired nucleotide bases, either those naturally occurring (such as adenine, guanine, thymine and cytosine), or other bases well known in the art, or may have modifications at the sugar or phosphate moieties to allow greater stability or greater complex formation to be achieved. In addition, such molecules may contain non-nucleotides in place of nucleotides. Such modifications are well known in the art, see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science, 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Sproat, B. *European Patent Application* 92110298.4 which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. All these publications are hereby incorporated by reference herein.

By "sufficient complementarity" is meant that sufficient base pairing occurs so that the R-loop base pair structure can be formed under the appropriate conditions to cause transcription of the enzymatic nucleic acid. Those in the art will recognize routine tests by which such sufficient base pairs can be determined. In general, between about 15 - 80 bases is sufficient in this invention.

By "physiological condition" is meant the condition in the cell or tissue to be targeted by the first nucleic acid molecule, although the R-loop complex may be formed under many other conditions. One example is use of a standard physiological saline at 37°C, but it is simply desirable in this invention that the R-loop structure exists to some extent at the site of action so that the expression of the desired nucleic acid will be achieved at that site of action. While it is preferred that the R-loop structure be stable under

- those conditions, even a minimal amount of formation of the R-loop structure to cause expression will be sufficient. Those in the art will recognize that measurement of such expression is readily achieved, especially in the absence of any promoter or leader sequence on the first nucleic acid molecule (Daube and von Hippel, 1992 Science 258, 1320). Such expression can thus only be achieved if an R-loop structure is truly formed with the second nucleic acid. If a promoter or leader sequence is provided, then it is preferred that the R-loop be formed at a site distant from those regions so that transcription is enhanced.
- 10 In a related aspect, the invention features a method for introduction of ribonucleic acid within a cell or tissue by forming an R-loop base-paired structure (as described above) with the first nucleic acid molecule lacking any promoter region or transcription termination signal such that once expression is initiated it will continue until the first nucleic acid is degraded.
- 15 In another related aspect, the invention features a method in which the second nucleic acid is provided with a localization factor, such as a protein, *e.g.*, an antibody, transferin, a nuclear localization peptide, or folate, or other such compounds well known in the art, which will aid in targeting the R-loop complex to a desired cell or tissue.
- 20 In preferred embodiments, the first nucleic acid is a plasmid, *e.g.*, one without a promoter or a transcription termination signal; the second nucleic acid is of length between about 40-200 bases and is formed of ribonucleotides at a majority of positions; and the second nucleic acid is covalently bonded with a ligand such as a nucleic acid, protein, peptide, lipid, carbohydrate, cellular receptor, nuclear localization factor, or is attached to maleimide or a thiol group: the first nucleic acid is an expression plasmid lacking a promoter able to express a desired gene, *e.g.*, it is a double-stranded molecule formed with a majority of deoxyribonucleic acids; the R-loop complex is a RNA/DNA heteroduplex;
- 25 no promoter or leader region is provided in the first nucleic acid; and the R-loop is adapted to prevent nucleosome assembly and is designed to aid recruitment of cellular transcription machinery.
- 30

In other preferred embodiments, the first nucleic acid encodes one or more enzymatic nucleic acids, *e.g.*, it is formed with a plurality of

intramolecular and intermolecular cleaving enzymatic nucleic acids to allow release of therapeutic enzymatic nucleic acid *in vivo*.

In a further related aspect, the invention features a complex of the above first nucleic acid molecules and second nucleic acid molecules.

5 R-loop complex

An R-loop complex is designed to provide a non-integrating plasmid so that, when an RNA polymerase binds to the plasmid, transcription is continuous until the plasmid is degraded. This is achieved by hybridizing an RNA molecule, 40 to 200 nucleotides in length, to a DNA expression
10 plasmid resulting in an R-loop structure (see figure 106). This RNA, when conjugated with a ligand that binds to a cell surface receptor, triggers internalization of the plasmid/RNA-ligand complex. Formation of R-loops in general is described by DeWet, 1987 Methods in Enzymol. 145, 235; Neuwald et al., 1977 J. Virol. 21,1019; and Meyer et al., 1986 J. Ult. Mol.
15 Str. Res. 96, 187. Thus, those in the art can readily design complexes of this invention following the teachings of the art.

Promoters placed in retroviral genomes have not always behaved as planned in that the additional promoter will serve as a stop signal or reverses the direction of the polymerase. Applicant was told that creation
20 of an R-loop between the promoter and the reporter gene increased the transfection efficiency. Incubation of an RNA molecule with a double-stranded DNA molecule, containing a region of complementarity with the RNA will result in the formation of a stable RNA-DNA hetroduplex and the DNA strand that has a sequence identical to the RNA will be displaced into
25 a loop-like structure called the R-loop. This displacement of DNA strand occurs because an RNA-DNA duplex is more stable compared to a DNA-DNA duplex. Applicant was also told that an 80 nt long RNA was used to generate a R-loop structure in a plasmid encoding the β -galactosidase gene. The R-loop was initiated either in the promoter region or in the
30 leader sequence. Plasmids containing an R-loop structure were microinjected into the cytoplasm of COS cells and the gene expression was assayed. R-loop formation in the promoter region of the plasmid inhibited expression of the gene. RNA that hybridized to the leader sequence between the promoter and the gene, or directly to the first 80
35 nucleotides of the mRNA increased the expression levels 8-10 fold. The

proposed mechanism is that R-loop formation prevents nucleosome assembly, thus making the DNA more accessible for transcription. Alternatively, the R-loop may resemble a RNA primer promoting either DNA replication or transcription (Daube and von Hippel, 1992, *supra*).

5 One of the salient features of this invention is to generate R-loops in expression vectors of choice and introduce them into cells to achieve enhanced expression from the expression vector. The presence of an R-loop may aid in the recruitment of cellular transcription machinery. Once an RNA polymerase binds to the plasmid and initiates transcription, the
10 process will continue until a termination signal is reached, or the plasmid is degraded.

 This invention will increase the expression of ribozymes inside a cell. The idea is to construct a plasmid with no transcription termination signal, such that a transcript-containing multiple ribozyme units can be
15 generated. In order to liberate unit length ribozymes, self-processing ribozymes can be cloned downstream of each therapeutic ribozyme (*see figure 107*) as described by Draper *supra*.

Ligand Targeting

 Another salient feature of this invention is that the RNA used to
20 generate R-loop structures can be covalently linked to a ligand (nucleic acid, proteins, peptides, lipids, carbohydrates, *etc.*). Specific ligands can be chosen such that the ligand can bind selectively to a desired cell surface receptor. This ligand-receptor interaction will help internalize a plasmid containing an R-loop. Thus, RNA is used to attach the ligand to the
25 DNA such that localization of the gene to certain regions of the cell is achieved. One of several methods can be used to attach a ligand to RNA. This includes the incorporation of deoxythymidine containing a 6 carbon spacer having a terminal primary amine into the RNA (*see figure 108*). This amino group can be directly derivatized with the ligand, such as folate (Lee and Low, 1994 *J. Biol. Chem.* 269, 3198-3204). The RNA containing a 6
30 carbon spacer with a terminal amine group is mixed with folate and the mixture is reacted with activators like 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). This reaction should be carried out in the presence of 1-Hydroxybenzotriazole hydrate (HOBT) to prevent
35 any undesirable side reactions.

The RNA can also be derivatized with a heterobifunctional crosslinking agent (or linker) like succinimidyl 4-(p-maleimidophenyl)butyrate (SMPB). The SMPB introduces a maleimide into the RNA. This maleimide can then react with a thiol moiety either in a peptide or in a protein. Thiols can also be introduced into proteins or peptides that lack naturally occurring thiols using succinylacetylthioacetate. The amino linker can be attached at the 5' end or 3' end of the RNA. The RNA can also contain a series of nucleotides that do not hybridize to the DNA and extend the linker away from the RNA/DNA complex, thus increasing the accessibility of the ligand for its receptor and not interfering with the hybridization. These techniques can be used to link peptides such as nuclear localization signal (NLS) peptides (Lanford et al., 1984 Cell 37, 801-813; Kalderon et al., 1984 Cell 39, 499-509; Goldfarb et al., 1986 Nature 322, 641-644) and/or proteins like the transferrin (Curiel et al., 1991 Proc. Natl. Acad. Sci. USA 88, 8850-8854; Wagner et al., 1992 Proc. Natl. Acad. Sci. USA 89, 6099-6103; Giulio et al., 1994 Cell. Signal 6, 83-90) to the ends of R-loop forming RNA in order to facilitate the uptake and localization of the R-loop-DNA complex. To link a protein to the ends of R-loop forming RNA, an intrinsic thiol can be used to react with the maleimide or the thiols can be introduced into the protein itself using either iminothiolate or succinimidyl acetyl thioacetate (SATA; Duncan et al., 1983 Anal. Biochem 132, 68). The SATA requires an additional deprotection step using 0.5 M hydroxylamine.

In addition liposomes can be used to cause an R-loop complex to be delivered to an appropriate intracellular site by techniques well known in the art. For example, pH-sensitive liposomes (Connor and Huang, 1986 Cancer Res. 46, 3431-3435) can be used to facilitate DNA transfection.

Calcium phosphate mediated or electroporation-mediated delivery of the R-loop complex into desired cells can also be readily accomplished.

30 In vitro Selection

In vitro selection strategies can be used to select nucleic acids that a) can form stable R-loops b) selectively bind to specific cell surface receptors. These nucleic acids can then be covalently linked to each other. This will help internalize the R-loop-containing plasmid efficiently using receptor-mediated endocytosis. The *in vitro* selection (evolution) strategy is

similar to approaches developed by Joyce (Beaudry and Joyce, 1992 Science 257, 635-641; Joyce, 1992 Scientific American 267, 90-97) and Szostak (Bartel and Szostak, 1993 Science 261:1411-1418; Szostak, 1993 TIBS 17, 89-93). Briefly, a random pool of nucleic acids is synthesized wherein each member contains two domains: a) one domain consists of a region with defined (known) nucleotide sequence; b) the second domain consists of a region with degenerate (random) sequence. The known nucleotide sequence domain enables: 1) the nucleic acid to bind to its target (a specific region of the double strand DNA), 2) complementary DNA (cDNA) synthesis and PCR amplification of molecules selected for their affinity to form R-loop and/or their ability to bind to a specific receptor, 3) introduction of a restriction endonuclease site for the purpose of cloning. The degenerate domain can be created to be completely random (each of the four nucleotides represented at every position within the random region) or the degeneracy can be partial (Beaudry and Joyce, 1992 Science 257, 635-641). In this invention, the degenerate domain is flanked by regions containing known sequences. This random library of nucleic acids is incubated under conditions that ensure equilibrium binding to either double-stranded DNA or cell surface receptor. Following incubation, nucleic acids are converted into complementary DNA (if the starting pool of nucleic acids is RNA). Nucleic acids with desired characteristics can be separated from the rest of the population of nucleic acids by using a variety of methods (Joyce, 1992 supra). The desired pool of nucleic acids can then be carried through subsequent rounds of selection to enrich the population with the most desired traits. These molecules are then cloned in to appropriate vectors. Recombinant plasmids can then be isolated from transformed bacteria and the identity of clones can be determined using DNA sequencing techniques.

Other embodiments are within the following claims.

TABLE ICharacteristics of Ribozymes**Group I Introns**

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNAseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figures 1 and 2)

Hairpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

Neurospora VS RNA Ribozyme

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.
Sequence requirements not fully determined.
Binding sites and structural requirements not fully determined. Only 1
known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table 2
Human ICAM HH Target sequence

nt. Position Target Sequences nt. Position Target Sequences

11	CCCCAGU C GACGCCUG	386	ACCGUGU A CUGGACTU
23	CUGAGCU C CCGGCTU	394	CUGGACTU C CAGAAGC
26	AGCUCCU C UGCUACTU	420	CACCCCU C CCGUCTU
31	CUCUGCU A CUCAGAG	425	CUCCCCU C UUGGCAG
34	UGCUACU C AGAGUUG	427	CCCCCUU U GGCAGCC
40	UCAGAGU U GCAACCU	450	AGAACCU U ACCCUAC
48	GCAACCU C AGCCUUG	451	GAAACCU A CCGUACG
54	UCAGCCU C GCUAUGG	456	UUAACCU A CGCUGCC
58	CCUGCCU A UGGCUUC	495	CCAACCU C ACCGUGG
64	UADGGCU C CCAGCAG	510	UGCUGCU C CGUGGGG
96	CCGCACU C CUGGUCC	564	CCGAGGU C ACCAACA
102	UCCUGGU C CUGCUUG	592	GAGAGAU C ACCAUGG
108	UCCUGCU C GGGGCCC	607	AGCCAAU U UCUGUG
115	CGGGGCU C UGUUCCC	608	GCCAAU U CUGUGGC
119	GCUCUGU U CCCAGGA	609	CCAATUU C UCGUGCC
120	CUCUGUU C CCAGGAC	611	AAUUCU C GGGCCGC
146	CAGACAU C UGUUCCC	656	GAGCUGU U UGAGAAC
152	UCUGUGU C CCCCACA	657	AGCUGUU U GAGAACA
158	UCCCCCU C AAAAGUC	668	AACACCU C GGGCCCC
165	CAAAAGU C ACCCUGC	677	GCCCCCU A CCAGCTC
168	AAGUCAU C CCGCCCC	684	ACCAGCU C CAGACCU
185	GGAGGCU C CGUGCUG	692	CAGACCU U UGUCCUG
209	AGCACTU C CUGUGAC	693	AGACCUU U GUCCUGC
227	CCCAAGU U GUUGGGC	696	CCUUGU C CUGCCAG
230	AAGUUGU U GGGCAUA	709	AGCGACU C CCCCACA
237	UGGGCAU A GAGACCC	720	CACAACU U GUCAGCC
248	ACCCCGU U GCUAAA	723	AACUUGU C AGCCCCC
253	GUUGCCU A AAAAGGA	735	CCCGGGU C CUGAGG
263	AAGGAGU U GCUCCUG	738	GGGUCCU A GAGGUGG
267	AGUUGCU C CCGCCUG	765	CCGUGGU C UGUUCCC
293	AAGGUGU A UGAACUG	769	GGUCUGU U CCGUGGA
319	AGAAGAU A GCAACCC	770	GUCUGUU C CCGGAC
335	AUGUGCU A UUCAAC	785	GGGCGU U CCGAGUC
337	GUGCUAU U CAAACUG	786	GGCUGUU C CCAGUCU
338	UGCUAUU C AAACCGC	792	UCCAGU C UCGAGG
359	GGSCAGU C AACAGCU	794	CCAGUCU C GGAGGCC
367	AACAGCU A AAACCUU	807	CCAGGU C CACUGG
374	AAAACCU U CUCACC	833	CAGAGGU U GAACCCC
375	AAACCUU C CUCACCG	846	CCACAGU C ACCUAG
378	CCUCCU C ACCGUGU	851	GUCACCU A UGGCAAC

863	AACGACU C CUGCUUG	1408	UCGAGAU C UUGAGGG
866	GACUCCU U CUUGGOC	1410	GAGAUUU U GAGGGCA
867	ACUCCUU C UUGGCCA	1421	GGCACCU A CCUCUGU
869	UCCUUCU C GGCCAAG	1425	CCUACCU C UGUCCGG
881	AAGGOCU C AGUCAGU	1429	CCUCUGU C GGGCCAG
885	CCUCAGU C AGUGUGA	1444	GAGCACU C AAGGGGA
923	GUGCAGU A AUACUGG	1455	GGGAGGU C ACCCGCG
936	CAGUAAU A CUGGGGA	1482	AUGUGCU C UCCCCC
978	UGACCAU C UACAGCU	1484	GUGCUUU C CCCCCG
980	ACCAUCU A CAGCUUU	1493	CCCCGGU A UGAGAUU
986	UACAGCU U UCCGGCG	1500	AUGAGAU U GUCAUCA
987	ACAGCUU U CCGGCGC	1503	AGADUGU C AUCAUCA
988	CAGCUUU C CCGGCGC	1506	UUGUCAU C AUCACUG
1005	ACGUGAU U CUGACGA	1509	UCAUCAU C ACUGUGG
1006	CGUGAUU C UGACGAA	1518	CUUGUGU A GCAGCCG
1023	CAGAGGU C UCAGAAG	1530	CGCAGU C AUAAUGG
1025	GAGGUCU C AGAAGGG	1533	CAGUCAU A AUGGGCA
1066	CCACCCU A GAGCCAA	1551	CAGGOCU C AGCACGU
1092	AUGGGGU U CCAGCCC	1559	AGCAOGU A CCUCUAU
1093	UGGGGUU C CAGCCCA	1563	CGUACCU C UAUAACC
1125	CCAGCUU C CUGCUGA	1565	UACUCUU A UAACCGC
1163	CGCAGCU U CUCCGCG	1567	CCUCUAU A ACCGCCA
1164	GCAGCUU C UCCUGCU	1584	GGAGAUU C AAGAAAU
1166	AGCUUCU C CUGCUCU	1592	AAGAAAU A CAGACTA
1172	UCCUGCU C UGCAACC	1599	ACAGACT A CAACAGG
1200	GCCAGCU U AUACACA	1651	CAGGOCU C CCUGAAC
1201	CCAGCUU A UACACAA	1661	UGAACCU A UCCCGGG
1203	AGCUUAU A CACAAGA	1663	AACCUAU C CCGGGAC
1227	GGGAGCU U CGUGUCC	1678	AGGGOCU C UUCUUG
1228	GGAGCUU C GUGUCCU	1680	GGCUCU U CCUCGCG
1233	UUGUGU C CUGUAUG	1681	GCCUCU C CUUGGCC
1238	GUCCUGU A UGGCCCC	1684	UCUUCUU C GGCCUUC
1264	EAGGGAU U GUCCGGG	1690	UCCGCCU U CCUAUAU
1267	GGAUUGU C CGGGAAG	1691	CGGCCUU C CCUAUAU
1294	AGAAAAU U CCAGACA	1696	UUCOCU A UUGGUGG
1295	GAAAAAU C CCAGCAG	1698	CCUAUAU U GGUGGCA
1306	GCAGACT C CAADUGG	1737	AAGACAU A UGCCADG
1321	CCAGGCU U GGGGGAA	1750	UGCAGCU A CACCUAC
1334	AACCCAU U GCCCGAG	1756	UACACCU A CCGGCC
1344	CCAGGCU C AAGUGUC	1787	AGGGCAU U GUCCUCA
1351	CAAGUGU C UAAAGGA	1790	GCADUGU C CUCAGUC
1353	AGUGUCU A AAGGAUG	1793	UUGUCCU C AGUCAGA
1366	UGGCACU U UCCACAU	1797	CCUCAGU C AGAUACA
1367	GGCACUU U CCACUG	1802	GUCAGAU A CAACAGC
1368	GCACUUU C CCACUGC	1812	ACAGCAU U UGGGGCC
1380	UGCCCAU C GGGGAUU	1813	CAGCAUU U GGGGCCA
1388	GGGGAAU C AGUGACU	1825	CCAUGGU A CCUGCAC
1398	UGACUGU C ACUGGAG	1837	CACACCU A AAACACU
1402	UGUCACU C GAGAUUU	1845	AAACACU A GGCCAGC

1856	CACGCAU C UGADCUG	2189	UADUUAU U GAGUGUC
1861	AUCUGAU C UGUAGUC	2196	UGAGUGU C UUUUADG
1865	GADUCUG A GUCACAU	2198	AGUGUCU U UUADGUA
1868	CUGUAGU C ACADGAC	2199	GUGUCUU U UADGUAG
1877	CAUGACU A AGCCAAG	2200	UGUCUUU U ADGUAGG
1901	CAAGACU C AAGACAU	2201	GUCUUUU A UGUAGGC
1912	ACAUGAU U GADGGAU	2205	UUUADGU A GGUUAAA
1922	UGGAGUG U AAAGUCU	2210	GUGGCUU A AAUGAAC
1923	GGADGUU A AAGGCUA	2220	UGAACAU A GGUUCUU
1928	UUAAGU C UAGCCUG	2224	CADAGGU C UUGGGCC
1930	AAAGUCU A GCCUGAU	2226	UAGGCUU C UGGCCUC
1964	GAGACAU A GCOCCAC	2233	CUGGCUU C ACGGAGC
1983	AGGACAU A CAACGGG	2242	CGGAGCU C CCGUCC
1996	GGGAAU A CUGAAAC	2248	UCCAGU C CAUGUCA
2005	UGAAAU U GCUGCCU	2254	UCCADU C ACADUCA
2013	GCUGCCU A UUGGGUA	2259	GUCACAU U CAAGGUC
2015	UGCCUUA U GGUUADG	2260	UCACAU C AAGGUCA
2020	AUUGGGU A UGUGAG	2266	UCRAGGU C ACCAGGU
2039	ACAGACU U ACAGAAG	2274	ACCAGGU A CAGUGU
2040	CAGACUU A CAGAAGA	2279	GUACAGU U GUACAGG
2057	UGGOCUU C CADAGAC	2282	CAGUGU A CAGGUG
2061	CCUCCAU A GACADGU	2288	UACAGGU U GUACACU
2071	CADUGU A GCADCAA	2291	AGGUUGU A CACGGCA
2076	GUAGCAU C AAAACAC	2321	AAAAGAU C AAADGGG
2097	CCACACU U CCGAAG	2338	UGGACU U CUCAUUG
2098	CACACUU C CUGAGGG	2339	GGGACUU C UCADUGG
2115	GCCAGCU U GGGCACU	2341	GACUUCU C AUUGGCC
2128	CUGUCUG C UACOGAC	2344	UUCUCAU U GGCCAAC
2130	GCUGUCU A CUGACCC	2358	CCUGCCU U UCCOCAG
2145	CAACCCU U GADGUA	2359	CUGCCUU U CCCCAGA
2152	UGADGAU A UGUADUU	2360	UGCCUUU C CCCCAGAA
2156	GAUADGU A UUUADUC	2376	GAGUGAU U UUUUUAU
2158	UAUGUAU U UAUUCAU	2377	AGUGAUU U UUCUADC
2159	AUGUAUU U AUUCAUU	2378	GUGAUUU U UCUAUUG
2160	UGUADUU A UUCAUUU	2379	UGAUUUU U CUADCGG
2162	UADUUAU U CAUUGU	2380	GADUUUU C UADGGC
2163	AUUUAUU C AUUGGUU	2382	UUUUUCU A UCGGCAC
2166	UAUUCAU U UGUUAUU	2384	UUUCUUA C GGCACAA
2167	AUUCAUU U GUUAUUU	2399	AAGCACU A UADGGAC
2170	CADUUGU U AUUUUAC	2401	GCACUUA A UGGACUG
2171	AUUUGUU A UUUUACC	2411	GACUGGU A AUGGUUC
2173	UUGUUAU U UUAACAG	2417	UAUUGGU U CACAGGU
2174	UGUUAUU U UAACAGC	2418	AAUGGUU C ACAGGUU
2175	GUUAUUU U ACCAGCU	2425	CACAGGU U CAGAGAU
2176	UUADUUU A CCAGCUA	2426	ACAGGUU C AGAGAUU
2183	ACCAGCU A UUUADUG	2433	CAGAGAU U ACCAGU
2185	CAGCUAU U UAUUGAG	2434	AGAGAUU A CCCCAGU
2186	AGCUAUU U AUUGAGU	2448	GAGGCUU U AUUCCUC
2187	GCUADUU A UUGAGUG	2449	AGGCUU A UUUCCUC

2451	GCCUUAU U CCUCCCU	2750	UADGUGU A GACAAGC
2452	CCUUAUU C CUCCCUU	2759	ACAAGCU C UCGCCCU
2455	UAUCCCU C CCUCCCU	2761	AAGCCCU C GCUCUGU
2459	CCUCCCU U CCCCCCA	2765	UCUCCCU C UGUCACC
2460	CUCCCUU C CCCCCAA	2769	GCUCUGU C ACCCAGG
2479	GACACCU U UGUUAGC	2797	GUGCAAU C AUGGUUC
2480	ACACCUU U GUUAGCC	2803	UCADGGU U CACUGCA
2483	CCUUGGU U AGCCACC	2804	CAUGGUU C ACUGCAG
2484	CUUUGUU A GCCACCU	2813	CUGCAGU C UUGACCU
2492	GCCACCU C CCCACCC	2815	GCAGUCU U GACCUUU
2504	CCCACAU A CAUUCUU	2821	UUGACCU U UUGGGCU
2508	CAUACAU U UCUGCCA	2822	UGACCUU U UGGGCCU
2509	AUAACAU U CUGCCAG	2823	GACCUUU U GGGCCCA
2510	UACAUUU C UGCCAGU	2829	UUGGGCU C AAGUGAU
2520	CCAGUGU U CACAATG	2837	AAGUGAU C CUCCACC
2521	CAGUGUU C ACAADGA	2840	UGAUCCU C CCACCCU
2533	UGACACU C AGCGGUC	2847	CCACCUU C AGCCUCC
2540	CAGCGGU C AUGUCUG	2853	UCAGCCU C CUGAGUA
2545	GUCAGU C UGGACAU	2860	CCUGAGU A GCUGGGA
2568	AGGGAUU A UGCCCAA	2872	GGACCAU A GGCCAC
2579	CCAAGCU A UGCCUUG	2877	AUAAGCU C ACAACAC
2585	UADGCCU U GUCCCUU	2899	GGCAAAU U UGADUUU
2588	GCCUUGU C CUUUGUU	2900	GCAAAAU U GAUUUUU
2591	UUGGCCU C UUGUCCU	2904	AUUUGAU U UUUUUUU
2593	GUCCCUU U GUCCUGU	2905	UUUGAUU U UUUUUUU
2596	CUUUGU C CUGUUUG	2906	UUGAUUU U UUUUUUU
2601	GUCCUGU U UGCAUUU	2907	UGAUUUU U UUUUUUU
2602	UCCUGUU U GCADUUC	2908	GAUUUUU U UUUUUUU
2607	UUUGCAU U UCACUGG	2909	AUUUUUU U UUUUUUU
2608	UUGCAUU U CACUGGG	2910	UUUUUUU U UUUUUUU
2609	UGCAUUU C ACUGGGA	2911	UUUUUUU U UUUUUUU
2620	GGGAGCU U GCACUAA	2912	UUUUUUU U UUUUUUC
2626	UUGCACU A UUGCAGC	2913	UUUUUUU U UUUUCCA
2628	GCACUAA U GCAGCUC	2914	UUUUUUU U UUUUCAG
2635	UGCAGCU C CAGUUUC	2915	UUUUUUU U UUUUCAG
2640	CUCCAGU U UCCUGCA	2916	UUUUUUU U UUCAGAG
2641	UCCAGUU U CCUGCAG	2917	UUUUUUU U UCAGAGA
2642	CCAGUUU C CUGCAGU	2918	UUUUUUU U CAGAGAC
2653	CAGUGAU C AGGGUCC	2919	UUUUUUU C AGAGACG
2659	UCAGGGU C CUGCAAG	2931	ACGGGGU C UOGCAAC
2689	CCAAGSU A UUGGAGG	2933	GGGGUCU C GCAACAU
2691	AAGGUUU U GGAGGAC	2941	GCAACAU U GCCCAGA
2700	GAGGACU C CCUCCCA	2951	CCAGACU U CCUUGGU
2704	ACUCCCU C CCAGCUU	2952	CAGACUU C CUUUGUG
2711	CCAGCUU U UGGAAGG	2955	ACUUCUU U UGUGUUA
2712	CCAGCUU U GGAAGGG	2956	CUUCCUU U GUGUAG
2721	GAAGGUU C AUCCGGG	2961	UUUGUGU U AGUUAUU
2724	GGGUCAU C CGGUGGU	2962	UUGUGUU A GUUAAUA
2744	UGUGUGU A UGUGUAG	2965	UGUUGU U AAUAAAG

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2966	GUAAGU A AUAAGC
2969	AGUAAU A AAGCUU
2975	UAAAGU U UCUAAC
2976	AAAGCU U CUCAACU
2977	AAGCUU C UCAACUG
2979	GCUUUCU C AACUGCC

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Table 3

Mouse ICAM HH Target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
11	CCUgGU C aCCUUG	367	AAUGGCU u cAAACCg
23	CaGuGgU u CUUGCU	374	gAAgCCU U CCUGcCC
26	UGGGuCU C UGUcCU	375	AAgCCU C CUgcCCc
31	CUUGCU c CUCCaca	378	CuacCaU C ACCGUGU
34	UuCUcaU a AGgGUcG	386	ACCGUGU A uUcGuU
40	gCAcAcU U GuAgCCU	394	CcGGACU u ucGauCu
48	aggAOCU C AGCCUGG	420	CACaCuU C CCCcCCg
54	UggGCCU C GuGADGG	425	CaOCCU C ccaGCAG
58	CaUgcCU u UaGUCC	427	CagCCU c aGCAGug
64	cAcccCU C CCAGCAG	450	AGgAOCU c ACCCUgC
96	CucugCU C CUGGcCC	451	GAAAcCU u uCCUuuG
102	UgCcaGU a CUGCUgG	456	UUAOCU c aGCcaCu
108	cuCUGCU c cuGGcCC	495	CuAcCaU C ACCGUGu
115	uGGuuCU C UGcUCCu	510	UGCUGCU C CGUGGGG
119	GgaaUGU c aCCAGGA	564	CUcAGGU a uCCauCc
120	CUUGCU C CugGcCC	592	GAAAGAU C ACaugGG
146	CAGuGgU C cGcuUCC	607	AGCCAAU U UCOCaUG
152	UCUGUGU C agCCaCu	608	GCCAAU U CUcUUGC
158	UCCuguU u AAAAacC	609	CCAAUuu C UCaUGCC
165	CgAAGU u gUuuUGC	611	AAUUCU C aUGOCCG
168	AAGcCuU C CUGCCCC	656	aAGCUGU U UGAGcug
185	GGUgGgU C CGUGCaG	657	AGCUGUU U GAGcugA
209	gcCAcuU C CUcUGgC	668	cyagCCU a GGCCaCC
227	CagAAGU U GUUuuGC	677	GaCCuCU A CCAGCCu
230	AAGUUGU U uuGUucc	684	uuCAGCU C CgGuCCU
237	UGuGcuU u GAGAcCu	692	CgCAcuU U cGauCUu
248	AaCCCaU c uCCUAAA	693	AGgaCcu c acCCUUGC
253	ccUGCCU A AggAaGA	696	CCUgUuU C CUGCCuc
263	AgGGuuU c uCUaCUG	709	gGCGgCU C CaCCuCA
267	AGggGCU C CUGCCUa	720	uACAACTU U uUCAGCu
293	AAGcUGU u UGAgCUG	723	AACTUuU C AGCuCCg
319	AGgAGAU A cugAgCC	735	aCCaGaU C CUgGAGa
335	cUGUGCU u UygAAC	738	uGGgCCU c GuGaUGG
337	GUcCaAU U CAcACUG	765	CaGUcGU C cGcUuCC
338	aGCUgUU u gAgCUGa	769	GGcCUGU U uCCUGcc
359	GuGCAGU C guCcGCU	770	uUuUGcu C CCGGAa
785	GGcCUGU U uCCuGcC	1353	AGUGggU c gAaGgUG
786	GcCUGUU u CCuGcCU	1366	UaaCAGU c UaCaACTU
792	UggagGU C UCGAaG	1367	aGCACcU c CCCACcu
794	CugGgCU u GGAGaCu	1368	GuACUgU a CCACUcu
807	CuGgGaU a uAOCUGG	1380	UGCCCAU C GGGgug
833	CAaAGcU c GAcacCC	1388	GGaGAcU C AGUGgCU
846	CCcugGU C ACCguUG	1398	UGgCUGU C ACagaAc
851	GagACCU c UacCAGC	1402	UGUgcuU u GAGAcCU

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863	AgCcACU u CcUCUgG	1408	gCGAGAU C ggGgaGG
866	GAagCCU U CcuGcCC	1410	GAGgUCU c GgaaGgg
867	AuUCgUU u cCGGagA	1421	ccCAccU A CuUuUGU
869	UCuUcCU C augCAAG	1425	aCUgCCU u gGUaGaG
881	AuGGCUU C AacCcGU	1429	uCUcUaU u GccCUuG
885	CCUugGU a gagGUGA	1444	GAaggCU C AgGaGGA
933	cUauAaU c ADuCUGG	1455	GGaAuGU C ACCaGga
936	uAaUcAU u CUGGuGc	1482	AgUUGuU u UgCuCCC
978	UAACagU C UACAaCU	1484	cUGuUCU u CCuCauG
980	ACagUCU A CAaCUUU	1493	CuguGcU u UGAGaAc
986	UACAaCU U DuCaGCU	1500	AUGAaAU c aUggUcc
987	ACAaCUU U uCaGCUc	1503	gGAcUaU a ADCADuc
988	CAaCUUU u CaGCUCC	1506	UUaUguU u AUaACcG
1005	ACCaGAU c CUGgaGA	1509	cuAcCAU C ACCgUGu
1006	uGaGagU C UGggGAA	1518	ucaUGGU c cCAGCG
1023	ugGAGGU C UCGAAG	1530	CuauAaU C AUucUGG
1025	GAGGUUU C gGAAGGG	1533	ugGUCAU u gUGGGCc
1066	CCAaCUU c aAaauAA	1551	CAUGCCU u AGCAgcU
1092	AcuGGaU c uCAGgCC	1559	AGCACcU c CCcaccU
1093	UGGaccU u CAGCCaA	1563	CuUAugU u UADAACC
1125	CCCAaCU C uUcuUGA	1565	UAguUuU A UAACCGC
1163	CGaAGCU U CUuuUGC	1567	ugUuUAU A ACCGCCA
1164	GaAGCUU C DUuUGCU	1584	GaAAGAU C AgGauAU
1166	AGCUUUU u uUGCUUU	1592	AgGAuAU A CAaguUA
1172	UCCUGuU u aaaAACC	1599	ACAaguU A CAgAAGG
1200	cuCuGCU c cUcCACA	1651	CcCaCCU C CCUGAgC
1201	gCuGCUU u UgaACAg	1661	gaAACCU u UCCuuuG
1203	AcuDUuU u CACcAGu	1663	AACCUuU C CunnGAa
1227	GGuAcaU a CGUGgC	1678	AGGaCCU C agCCUgG
1228	GaAGCUU C uDuUgCU	1680	aGCCaCU U CCUCuGg
1233	UUCGUuU C CgGagaG	1681	GCCaCUU C CUUGgC
1238	GUgCUGU A UGUuCCu	1684	aCUUCCU C uGgCUgu
1264	GAaGGgU c GUgCaaG	1690	cCGGaCU U uCgAUcU
1267	uGAgagU C uGGGgAA	1691	CGGaCCU u CgAUcUU
1294	AGgAgAU a CugAGCc	1696	UgCCCAU c ggGcUGG
1295	GAggggU C uCAGCAG	1698	CggAUAU a ccUGGag
1306	GCAGACU C ugAaaUG	1737	gAGACcU c UAACAgc
1321	gaAGGCU c aGGAgGA	1750	gGCGGCU c CAOCUca
1334	AAOCCAU c uCCuaAa	1756	gAagCCU u CCUGGCC
1344	auGAGCU C gAGaGUg	1787	gaGaCAU U GUCCcCA
1351	ugAaUGU a UAAguuA	1790	GCAUUGU u CUUuaau
1793	UgGUCCU C gGcugGA	2173	UUagagU U UUAOCAG
1797	CacCAGU C ACAUAaA	2174	UagagUU U UAOCAGC
1802	acCAGAU c CuggAGa	2175	agagUUU U ACCAGCU
1812	ACuGgAU c UcaGGCC	2176	gagUUUU A CCAGCUA
1813	CAGCAUU U acccuCA	2183	ACCAGCU A UUUADUG
1825	CCAcGcU A CCUCugC	2185	CAGCUAU U UAUGAG
1837	CAugCCU u uAgCuCc	2186	AGCUAUU U AUUGAGU
1845	cgAgcCU A GGCCACc	2187	GCUAUUU A UUGAGUA

1856	CggaCuU u cGADCUu	2189	UAUUUAU U GAGUacC
1861	AcaUGAU a UccAGUa	2196	caAcUcU u cUUGAUG
1865	cAcuUGU A GcCuCag	2198	gcaGcCU c UUAUGUu
1868	CaccAGU C ACADaAa	2199	GccUCCU a UgUuUAu
1877	CAUGcCU u AGCagcu	2200	UcUuccU c AUGcAaG
1901	uAAaACU C AAGggAc	2201	aagUUUU A UGUcGGC
1912	AuAUagU a GAUcagU	2205	UUUAUGU c GGCcugA
1922	UGaADGU a uAAGUua	2210	GgAGaCU c AgUGgcu
1923	uGAUGcU c AgGUaUc	2220	cuggCAU u GuUCCCU
1928	UUAgAGU u UuaCCaG	2224	CucAGGU a UCCauCC
1930	AgAGUuU u aCCAGcU	2226	UgGaUcU C aGGCCgC
1964	GAGACAU u GuCCCCa	2233	CUGaCCU C cuGGAGg
1983	AGGAuAU A CAAGUua	2242	uGGAGCU a gCgGaCC
1996	aGGAgAU A CUGAgcC	2248	UauCcaU C CAUccCA
2005	UGgAgCU a GCGGaCc	2254	UCCAuU C ACaCUgA
2013	GUUauU A UUGaGUA	2259	aUCACAU U CAcGGUg
2015	UGCCcAU c GGGgugG	2260	UCACAUU C AcGGUgc
2020	ggUGGuU c UuCUGAG	2266	ggAAuGU C ACCAGGa
2039	gCuGgCU a gCAGAgG	2274	ACCAGaU c CuGgaGa
2040	CuGACcU c CuGgAGg	2279	GaAggGU c GUgCaAG
2057	UGcuCCU C CAcAucC	2282	aAGcUGU u ugagcUG
2061	CuaCCAU c acCgUGU	2288	UauAaGU U aUggcCU
2071	CACuUGU A GCCcCAG	2291	caGUgGU u CuCUGUu
2076	GUAGCcU C AgAgCua	2321	gAAAGAU C AcADGGG
2097	CaACuCU U CuUGAuG	2338	UGaGACU c CUgcccUG
2098	CACACU C CcccCcG	2339	GaaACcU u UCCUUuG
2115	GCCAGCU c GGaggaU	2341	GACcUcU a ccaGcCu
2128	CaGCUaU u UAUUGAG	2344	UUuccAU c uuCCAgC
2130	cCUGUuU c CUgCcUc	2358	CCcagCU c UCcagCAG
2145	CRAcuCU U cuUGAdg	2359	CUGUuU U gaaCAGA
2152	UauUaAU u UagAgUU	2360	aaCCUUU C CUuuGAA
2156	uugAUGU A UUUAUUa	2376	agGUGgU U cUUUdga
2158	gAUGUAU U UAUUaAU	2377	ggUGgUU c UUCUgag
2159	AUGUAU U AUUaAUU	2378	agGgUUU c UCUAcuG
2160	UGUAUU A UUAaUUU	2379	UGcUUUU c ucAUaaG
2162	UAUUUAU U aAUUUag	2380	aAgUUUU a UgUCCGC
2163	AUGUAU u AUUaaUu	2382	aUUcUcU A UuGcCcC
2166	acUUCAU U cucUAUU	2384	aUcCagU a GaCACAA
2167	AUguAUU U aUUaAUU	2399	AAaCAU A UgUGGAC
2170	uAUUUaU U AaUUUAU	2401	aagCUgU u UGagCUG
2171	AgUUUUU u UgcUcCC	2411	uACUGGU c AgGaUgC
2417	gAAUGGU a CAuAcGU	2691	AAuGUcU c cEAGGcC
2418	AcUGGaU C uCAGGcc	2700	GAAgCCU u CCUgCCC
2425	CAugGGU c gAggGuU	2704	gacCuCU a CCAGCcU
2426	AuuaaUU u AGAGUUU	2711	CCCAGCU c UcagcaG
2433	uAGAGuU U uaCCAGc	2712	gagGuCU c GGAAGGG
2434	AGAGUUU u aCCAGcu	2721	GAAGGGU C gUgCaAG
2448	GAAGCCU U ccUgCcC	2724	GGuaCAU a CGUGUGc
2449	AaGCCUU c cUgCccC	2744	gGUGgGU c cGUGcAG

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2451	GCCUguU U	CCUgCCU	2750	UAUuUaU u	GAguaCC
2452	CCUguUU C	CUgCCUc	2759	cCggaCU u	UCGaUCCU
2455	gAagCCU u	CCUgCCC	2761	AgGacCU C	aCCcUGc
2459	CCaCaCU U	CCCCCcc	2765	UuUuGCU C	UGcCgCu
2460	CaCaCCU C	CCCCCcg	2769	agUCCGU C	AaaCAGG
2479	GAgACCU c	UaccAGC	2797	aUGaAAU C	AUGGUcC
2480	uCACCGU U	GUgAuCC	2803	UCAUGGU c	CcagGCC
2483	CCaaUGU c	AGCCACC	2804	ggUGGgU c	cgUGCAG
2484	CUUuUUU c	aCCAguc	2813	CUcCgGU C	cUGACCc
2492	agCACCU C	CCCACCu	2815	aCAGUCC a	cAaCUUU
2504	CCCACcU A	CuUUUGU	2821	cUGACCU c	cUGGagg
2508	uAUcCAU c	caUcCCA	2822	gGAgCcU c	cGGcCUu
2509	uUAgAgU U	uUaCCAG	2823	ugCCUUU a	GcuCCcA
2510	UAgAgUU U	UaCCAGc	2829	cUGGaCU a	uAaUcAU
2520	CuuuUGU U	CcCAADG	2837	AgGUGgU u	CUuCuGa
2521	CAGcaUU u	ACccUcA	2840	UGagaCU C	CugCCUg
2533	UGAugCU C	AGguuUC	2847	CCaAugU C	AGCCaCC
2540	CAGCaGU C	cgcUGUG	2853	gCAGCCU C	uUauGUu
2545	GUgcUGU a	UGGuCcU	2860	gCcaAGU A	aCUGuGA
2568	guGaAgU c	UGuCaAA	2872	GGACCUU c	aGCcaAg
2579	auAAGuU A	UGgCcUG	2877	uUccGCCU a	cCAuCAC
2585	cugGCaU U	GUuCUUU	2899	cGgAcuU U	cGADcUU
2588	GCaUUUU u	CUUaaU	2900	uuAAuUU a	GAgUUUU
2591	UGGUuCU C	UgcUUUU	2904	AcUUcAU U	cUcUaUU
2593	cUuCUuU U	GcuUGCc	2905	cUUCAUU c	UcUaUUG
2596	CUuUUUU u	CccaaUG	2906	UUGAUgU a	UUUaUUa
2601	acCgUGU a	UuCGUUU	2907	UGuaUUU a	UUaaUUU
2602	UCCaGcU a	cCAUccC	2908	GAgcGUU c	UUUUgcU
2607	cUcGgAU a	UacCUGG	2909	AgcUUcU U	UUgcUcU
2608	caGCAgU c	CgCUGuG	2910	UgUaUUU a	UUaaUUU
2609	gGaAUgU C	ACcaGGA	2911	UgUaUUU a	UUaaUUU
2620	aGGAcCU c	aCCcUGc	2912	UUgUUcU c	UaaUgUC
2626	UUuCGaU c	UUcCAGC	2913	UUUcUcU a	cUggUCA
2628	GCAcAcU U	GuAGCcu	2914	UgcUUUU c	UcaUaAG
2635	UuCAGCU C	CgGUccu	2915	aUUUaUU a	aUUuAGA
2640	ggCCuGU U	UCCUGCc	2916	UaUUcgU U	UcCgGAG
2641	cCCAGcU c	uCaGCAG	2917	aUUcgUU U	cCgGAGA
2642	CCuGUUU C	CUUGCuc	2918	UUcgUUU c	CgGAGAg
2653	uAcUGgU c	AGGaUgC	2919	UUcUcaU a	AGgGuCC
2659	gaAGGUU C	gUGCAAG	2931	ugGaGGU C	UCGgAAG
2689	CuAAuGU c	UccGAGG	2933	GaGGUCU C	GgAAggg
2941	GagACAU U	GuCCccA			
2951	CCAcyCU a	CCUcUGc			
2952	CAGcagU C	CgcUGUG			
2955	AgUgaCU c	UGUGUcA			
2956	uUUCCUU U	GaaUcAa			
2961	UcUGUGU c	AGccAcU			
2962	aUGUaUU u	aUUAAUu			
2965	UUUGAaU c	AAUAAAG			

2966	GcUgGcU A gcAgAGg
2969	aaUcAAU A AAGuUUU
2975	UAgAGuU U UacCAgC
2976	gAgGgUU U CUCuACTU
2977	AAGCUgU u UgAgCUG
2979	uCaUUU C uAuUGCC

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Table 4
Human ICAM HH Ribozyme Sequences

nt. Position	Ribozyme Sequence
11	CAGGUC CUGAUGAGGCCGAAAGGCCGAA ACUGGGG
23	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
26	AGUAGCA CUGAUGAGGCCGAAAGGCCGAA AGGAGCU
31	CUCUGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGAG
34	CACUCU CUGAUGAGGCCGAAAGGCCGAA AGUAGCA
40	AGGUUC CUGAUGAGGCCGAAAGGCCGAA ACUCUGA
48	CGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGUUC
54	CCAUAGC CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
58	GGAGCCA CUGAUGAGGCCGAAAGGCCGAA AGCGAGG
64	CUGCUGG CUGAUGAGGCCGAAAGGCCGAA AGCCAU
96	GGACCAG CUGAUGAGGCCGAAAGGCCGAA AGUGCGG
102	CGAGCAG CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
108	GAGCCCC CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
115	GGGAACA CUGAUGAGGCCGAAAGGCCGAA AGCCCCG
119	UCCUGGG CUGAUGAGGCCGAAAGGCCGAA ACAGAGC
120	GUCUUGG CUGAUGAGGCCGAAAGGCCGAA AACAGAG
146	GGACACA CUGAUGAGGCCGAAAGGCCGAA AUGUCUG
152	UGAGGGG CUGAUGAGGCCGAAAGGCCGAA ACACAGA
158	GACUUUU CUGAUGAGGCCGAAAGGCCGAA AGGGGGA
165	GCAGGAU CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
168	GGGCGAG CUGAUGAGGCCGAAAGGCCGAA AUGACUU
185	CAGCACG CUGAUGAGGCCGAAAGGCCGAA AGCCUCC
209	GUCACAG CUGAUGAGGCCGAAAGGCCGAA AGGUGCU
227	GCCCCAC CUGAUGAGGCCGAAAGGCCGAA ACUUGGG
230	UAUGCCC CUGAUGAGGCCGAAAGGCCGAA ACAACUU
237	GGGUCUC CUGAUGAGGCCGAAAGGCCGAA AUGCCCA
248	UUUAGGC CUGAUGAGGCCGAAAGGCCGAA ACCGGGU
253	UCCUUUU CUGAUGAGGCCGAAAGGCCGAA AGGCAAC
263	CAGGAGC CUGAUGAGGCCGAAAGGCCGAA ACUCCUU
267	CAGGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAACU
293	CAGUUCA CUGAUGAGGCCGAAAGGCCGAA ACACCUU
319	GGUUGGC CUGAUGAGGCCGAAAGGCCGAA ADCUUUU
335	GUUGGAA CUGAUGAGGCCGAAAGGCCGAA AGCACAU
337	CAGUUUG CUGAUGAGGCCGAAAGGCCGAA AUGACAC
338	GCAGUUU CUGAUGAGGCCGAAAGGCCGAA AAUAGCA
359	AGCUGUU CUGAUGAGGCCGAAAGGCCGAA ACUGCCC
367	AAGGUUU CUGAUGAGGCCGAAAGGCCGAA AGCUGUU
374	GGUGAGG CUGAUGAGGCCGAAAGGCCGAA AGGUUUU
375	CGUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUUU
378	ACACGGU CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
386	AGUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACGGU
394	CGUUCUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
420	AAGAGGG CUGAUGAGGCCGAAAGGCCGAA AGGGGUG
425	CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AGGGGAG

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427	GGCUGCC	CUGADGAGGCGAAAGGCGGAA	AGAGGGG
450	GUAGGGU	CUGADGAGGCGAAAGGCGGAA	AGGUUCU
451	CGUAGGG	CUGADGAGGCGAAAGGCGGAA	AAGGUC
456	GGCAGCG	CUGADGAGGCGAAAGGCGGAA	AGGUAA
495	CCACGGU	CUGADGAGGCGAAAGGCGGAA	AGGUUGG
510	CCCCACG	CUGADGAGGCGAAAGGCGGAA	AGCAGCA
564	UGGUUGU	CUGADGAGGCGAAAGGCGGAA	ACCUACG
592	CCAUGGU	CUGADGAGGCGAAAGGCGGAA	ADUCUC
607	CACGAGA	CUGADGAGGCGAAAGGCGGAA	ADUGGCU
608	GCACGAG	CUGADGAGGCGAAAGGCGGAA	AAUUGGC
609	GGCACEA	CUGADGAGGCGAAAGGCGGAA	AAAUUGG
611	GCGGCAC	CUGADGAGGCGAAAGGCGGAA	AGAAADU
656	GUUCUCA	CUGADGAGGCGAAAGGCGGAA	ACAGCUC
657	UGUUCUC	CUGADGAGGCGAAAGGCGGAA	AACAGCU
668	GGGGGCC	CUGADGAGGCGAAAGGCGGAA	AGGUGUU
677	GAGCUGG	CUGADGAGGCGAAAGGCGGAA	AGGGGGC
684	AGGUUCG	CUGADGAGGCGAAAGGCGGAA	AGCUGGU
692	CAGGACA	CUGADGAGGCGAAAGGCGGAA	AGGUUCG
693	GCAGGAC	CUGADGAGGCGAAAGGCGGAA	AAGGUUC
696	CUGGCAG	CUGADGAGGCGAAAGGCGGAA	ACAAAGG
709	UGUGGGG	CUGADGAGGCGAAAGGCGGAA	AGUUGCU
720	GGCUGAC	CUGADGAGGCGAAAGGCGGAA	AGUUGUG
723	GGGGGCU	CUGADGAGGCGAAAGGCGGAA	ACAAGUU
735	CCUCUAG	CUGADGAGGCGAAAGGCGGAA	ACCGGGG
738	CCACCUU	CUGADGAGGCGAAAGGCGGAA	AGGACCC
765	GGGAACA	CUGADGAGGCGAAAGGCGGAA	ACCAAGG
769	UCCAGGG	CUGADGAGGCGAAAGGCGGAA	ACAGACC
770	GUCCAGG	CUGADGAGGCGAAAGGCGGAA	AACAGAC
785	GACUGGG	CUGADGAGGCGAAAGGCGGAA	ACAGCCC
786	AGACUGG	CUGADGAGGCGAAAGGCGGAA	AACAGCC
792	CCUCCGA	CUGADGAGGCGAAAGGCGGAA	ACUGGCA
794	GGCCUCC	CUGADGAGGCGAAAGGCGGAA	AGACUGG
807	CCAGGUG	CUGADGAGGCGAAAGGCGGAA	ACCUGGG
833	GGGGUUC	CUGADGAGGCGAAAGGCGGAA	ACCUUCG
846	CAUAGGU	CUGADGAGGCGAAAGGCGGAA	ACUGUGG
851	GUUGCCA	CUGADGAGGCGAAAGGCGGAA	AGGUGAC
863	CGAGAAG	CUGADGAGGCGAAAGGCGGAA	AGUUGUU
866	GGCCGAG	CUGADGAGGCGAAAGGCGGAA	AGGAGUC
867	UGGCCGA	CUGADGAGGCGAAAGGCGGAA	AAGGAGU
869	CUUGGOC	CUGADGAGGCGAAAGGCGGAA	AGAAGGA
881	ACUGACU	CUGADGAGGCGAAAGGCGGAA	AGGCCUU
885	UCACACU	CUGADGAGGCGAAAGGCGGAA	ACUGAGG
933	CCAGUAT	CUGADGAGGCGAAAGGCGGAA	ACUGCAC
936	UCCCCAG	CUGADGAGGCGAAAGGCGGAA	AUUAUCG
978	AGCUGUA	CUGADGAGGCGAAAGGCGGAA	AUGGUCA
980	AAAGCUG	CUGADGAGGCGAAAGGCGGAA	AGADGGU
986	CGCCGGA	CUGADGAGGCGAAAGGCGGAA	AGCUGUA
987	GGCCCGG	CUGADGAGGCGAAAGGCGGAA	AAGCUGU
988	GGCCCGG	CUGADGAGGCGAAAGGCGGAA	AAAGCUG

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1005	UCGUCAG	CUGAUGAGGCGAAAGGCGGAA	AUCACGU
1006	UUCGUGA	CUGAUGAGGCGAAAGGCGGAA	AADACAG
1023	CUUCUGA	CUGAUGAGGCGAAAGGCGGAA	ACUCUG
1025	COCUCU	CUGAUGAGGCGAAAGGCGGAA	AGACCU
1066	UGGCU	CUGAUGAGGCGAAAGGCGGAA	AGGUGG
1092	GGGCU	CUGAUGAGGCGAAAGGCGGAA	ACCCAU
1093	UGGCU	CUGAUGAGGCGAAAGGCGGAA	AACCCA
1125	UCAGCAG	CUGAUGAGGCGAAAGGCGGAA	AGCUGG
1163	GCAGGAG	CUGAUGAGGCGAAAGGCGGAA	AGCUGG
1164	AGCAGGA	CUGAUGAGGCGAAAGGCGGAA	AAGCUG
1166	AGAGCAG	CUGAUGAGGCGAAAGGCGGAA	AGAAGCU
1172	GGUUGCA	CUGAUGAGGCGAAAGGCGGAA	AGCAGGA
1200	UGUGUAT	CUGAUGAGGCGAAAGGCGGAA	AGCUGG
1201	UGUGUA	CUGAUGAGGCGAAAGGCGGAA	AAGCUG
1203	UCUUGUG	CUGAUGAGGCGAAAGGCGGAA	AUAAGCU
1227	GGACAG	CUGAUGAGGCGAAAGGCGGAA	AGCUCU
1228	AGGACAC	CUGAUGAGGCGAAAGGCGGAA	AAGCUC
1233	CAUACAG	CUGAUGAGGCGAAAGGCGGAA	ACAAGAA
1238	GGGGCCA	CUGAUGAGGCGAAAGGCGGAA	ACAGGAC
1264	CCGGAC	CUGAUGAGGCGAAAGGCGGAA	AUCCUC
1267	UUUCCCG	CUGAUGAGGCGAAAGGCGGAA	ACAUCU
1294	UGCUGGG	CUGAUGAGGCGAAAGGCGGAA	AUUUCU
1295	CUCUGG	CUGAUGAGGCGAAAGGCGGAA	AUUUUC
1306	CACAUUG	CUGAUGAGGCGAAAGGCGGAA	AGUCUG
1321	UUCCCC	CUGAUGAGGCGAAAGGCGGAA	AGCUGG
1334	CUUGGGC	CUGAUGAGGCGAAAGGCGGAA	ADGGGU
1344	GACACUU	CUGAUGAGGCGAAAGGCGGAA	AGCUCG
1351	UCCUUUA	CUGAUGAGGCGAAAGGCGGAA	ACAUCU
1353	CAUCCUU	CUGAUGAGGCGAAAGGCGGAA	AGACAU
1366	AGUGGA	CUGAUGAGGCGAAAGGCGGAA	AGUGCA
1367	CAGUGGG	CUGAUGAGGCGAAAGGCGGAA	AAGUGC
1368	GCAGUGG	CUGAUGAGGCGAAAGGCGGAA	AAAGUG
1380	ADUCCC	CUGAUGAGGCGAAAGGCGGAA	ADUGGC
1388	AGUCACU	CUGAUGAGGCGAAAGGCGGAA	ADUCCC
1398	CUUGAGU	CUGAUGAGGCGAAAGGCGGAA	ACAGUA
1402	AGAUUC	CUGAUGAGGCGAAAGGCGGAA	AGUGAC
1408	CCUCAA	CUGAUGAGGCGAAAGGCGGAA	ADUCUA
1410	UGCCUUC	CUGAUGAGGCGAAAGGCGGAA	AGAUUC
1421	ACAGAGG	CUGAUGAGGCGAAAGGCGGAA	AGGUGC
1425	CCGACA	CUGAUGAGGCGAAAGGCGGAA	AGGUAG
1429	CUGGCC	CUGAUGAGGCGAAAGGCGGAA	ACAGAG
1444	UCCCUU	CUGAUGAGGCGAAAGGCGGAA	AGUGUC
1455	CGGGGU	CUGAUGAGGCGAAAGGCGGAA	ACUCCC
1482	GGGGGA	CUGAUGAGGCGAAAGGCGGAA	AGCACU
1484	CGGGGG	CUGAUGAGGCGAAAGGCGGAA	AGAGAC
1493	AAUCUA	CUGAUGAGGCGAAAGGCGGAA	ACCGGG
1500	UGAUGAC	CUGAUGAGGCGAAAGGCGGAA	ADUCAU
1503	UGAUGAU	CUGAUGAGGCGAAAGGCGGAA	ACAUCU
1506	CAGUGAU	CUGAUGAGGCGAAAGGCGGAA	AUGCAA

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1509	CCACAGU	CUGAUGAGGCGGAAAGGCGGAA	ADGADGA
1518	CGGCUGC	CUGAUGAGGCGGAAAGGCGGAA	ACACACAG
1530	CCAUUAU	CUGAUGAGGCGGAAAGGCGGAA	ACUGCGG
1533	UGCCCAU	CUGAUGAGGCGGAAAGGCGGAA	ADGACUG
1551	ACGUGCU	CUGAUGAGGCGGAAAGGCGGAA	AGGCGUG
1559	AUAGAGG	CUGAUGAGGCGGAAAGGCGGAA	ACGUGCU
1563	GGUUAUA	CUGAUGAGGCGGAAAGGCGGAA	AGGUACG
1565	GCGGUUA	CUGAUGAGGCGGAAAGGCGGAA	AGAGGUA
1567	UGGCGGU	CUGAUGAGGCGGAAAGGCGGAA	AUAGAGG
1584	AUUUCUU	CUGAUGAGGCGGAAAGGCGGAA	AUCUCC
1592	UAGUCUG	CUGAUGAGGCGGAAAGGCGGAA	AUUUCUU
1599	CCUGUUG	CUGAUGAGGCGGAAAGGCGGAA	AGUCUGU
1651	GUUCAGG	CUGAUGAGGCGGAAAGGCGGAA	AGGCGUG
1661	CCGGGA	CUGAUGAGGCGGAAAGGCGGAA	AGGUUCA
1663	GUCGCGG	CUGAUGAGGCGGAAAGGCGGAA	AUAGGUU
1678	CGAGGAA	CUGAUGAGGCGGAAAGGCGGAA	AGGCCCU
1680	GCCGAGG	CUGAUGAGGCGGAAAGGCGGAA	AGAGGCC
1681	GGCCGAG	CUGAUGAGGCGGAAAGGCGGAA	AAGAGGC
1684	GAAGGCC	CUGAUGAGGCGGAAAGGCGGAA	AGGAGA
1690	AUAUGGG	CUGAUGAGGCGGAAAGGCGGAA	AGGCCGA
1691	AUAUGG	CUGAUGAGGCGGAAAGGCGGAA	AAGGCCG
1696	CCACCAA	CUGAUGAGGCGGAAAGGCGGAA	AUGGGAA
1698	UGCCACC	CUGAUGAGGCGGAAAGGCGGAA	AUAUGGG
1737	CAUGGCA	CUGAUGAGGCGGAAAGGCGGAA	AUGUCUU
1750	GUAGGUG	CUGAUGAGGCGGAAAGGCGGAA	AGUCUCA
1756	GGGCCGG	CUGAUGAGGCGGAAAGGCGGAA	AGGUGUA
1787	UGAGGAC	CUGAUGAGGCGGAAAGGCGGAA	AUGCCCU
1790	GACUGAG	CUGAUGAGGCGGAAAGGCGGAA	ACAAGGC
1793	UCUGACU	CUGAUGAGGCGGAAAGGCGGAA	AGGACAA
1797	UGUAUCU	CUGAUGAGGCGGAAAGGCGGAA	ACUGAGG
1802	GCUGUUG	CUGAUGAGGCGGAAAGGCGGAA	AUCUGAC
1812	GGCCCCA	CUGAUGAGGCGGAAAGGCGGAA	AUGCUGU
1813	UGGCCCC	CUGAUGAGGCGGAAAGGCGGAA	AAUGCUG
1825	GUGCAGG	CUGAUGAGGCGGAAAGGCGGAA	ACCAUGG
1837	AGUGUUU	CUGAUGAGGCGGAAAGGCGGAA	AGGUGUG
1845	CGUGGCC	CUGAUGAGGCGGAAAGGCGGAA	AGUGUUU
1856	CAGAUCA	CUGAUGAGGCGGAAAGGCGGAA	ADGCGUG
1861	GACTACA	CUGAUGAGGCGGAAAGGCGGAA	AUCAGAU
1865	AUGUGAC	CUGAUGAGGCGGAAAGGCGGAA	ACAGAUU
1868	GUCAUGU	CUGAUGAGGCGGAAAGGCGGAA	ACUACAG
1877	CUUGGCU	CUGAUGAGGCGGAAAGGCGGAA	AGUCADG
1901	AUGUCUU	CUGAUGAGGCGGAAAGGCGGAA	AGUCUUG
1912	AUCCAUU	CUGAUGAGGCGGAAAGGCGGAA	AUCAUGU
1922	AGACUUU	CUGAUGAGGCGGAAAGGCGGAA	ACAUCCA
1923	UAGACUU	CUGAUGAGGCGGAAAGGCGGAA	AACAUCC
1928	CAGGCUA	CUGAUGAGGCGGAAAGGCGGAA	ACUUUAA
1930	AUCAGGC	CUGAUGAGGCGGAAAGGCGGAA	AGACUUU
1964	GUGGGGC	CUGAUGAGGCGGAAAGGCGGAA	AUGUCUC
1983	CCAGUUG	CUGAUGAGGCGGAAAGGCGGAA	AUGUCUU

1996	GUUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUUUCCC
2005	AGGCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCA
2013	UACCCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGC
2015	CATAACC	CUGAUGAGGCCGAAAGGCCGAA	ADTAGCA
2020	CUCAGCA	CUGAUGAGGCCGAAAGGCCGAA	ACCCAAU
2039	CUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGU
2040	UCUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUG
2057	GUCUADG	CUGAUGAGGCCGAAAGGCCGAA	AGGGCCA
2061	ACAUGUC	CUGAUGAGGCCGAAAGGCCGAA	ADGGAGG
2071	UUGAUGC	CUGAUGAGGCCGAAAGGCCGAA	ACACAUG
2076	GGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	ADGCUAC
2097	CGUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGG
2098	CCGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2115	AGUGCCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
2128	GUCAGUA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAG
2130	GGGUCAG	CUGAUGAGGCCGAAAGGCCGAA	AGACAGC
2145	UADCAUC	CUGAUGAGGCCGAAAGGCCGAA	AGGGUUG
2152	AAAUACA	CUGAUGAGGCCGAAAGGCCGAA	ADCAUCA
2156	GAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUADC
2158	ADGAADA	CUGAUGAGGCCGAAAGGCCGAA	ADACADA
2159	AADGAAT	CUGAUGAGGCCGAAAGGCCGAA	AADACAT
2160	AAAUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2162	ACAAADG	CUGAUGAGGCCGAAAGGCCGAA	ADAAADA
2163	AACAAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAU
2166	AAUAACA	CUGAUGAGGCCGAAAGGCCGAA	ADGAADA
2167	AAAUAC	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAU
2170	GUAAAAU	CUGAUGAGGCCGAAAGGCCGAA	ACAAADG
2171	GGUAAAA	CUGAUGAGGCCGAAAGGCCGAA	AACAAAU
2173	CGGUAAA	CUGAUGAGGCCGAAAGGCCGAA	AUAACAA
2174	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AAUAACA
2175	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAC
2176	UAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAADAA
2183	CAADAAA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGU
2185	CUCAADA	CUGAUGAGGCCGAAAGGCCGAA	ADAGCUG
2186	ACTCAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGCU
2187	CAUCUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2189	GACACUC	CUGAUGAGGCCGAAAGGCCGAA	ADAAADA
2196	CAUAAA	CUGAUGAGGCCGAAAGGCCGAA	ACACUCA
2198	UACAUAA	CUGAUGAGGCCGAAAGGCCGAA	AGACACU
2199	CUACADA	CUGAUGAGGCCGAAAGGCCGAA	AAGACAC
2200	CCUACAU	CUGAUGAGGCCGAAAGGCCGAA	AAAGACA
2201	GCCUACA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGAC
2205	UUUAGCC	CUGAUGAGGCCGAAAGGCCGAA	ACAUAAA
2210	GUUCADU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUAC
2220	AGAGACC	CUGAUGAGGCCGAAAGGCCGAA	ADGUUCA
2224	GGCCAGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUADG
2226	GAGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGACCTA
2233	GCUCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCAG
2242	GGACUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCUCUG

2248	UGACAUG	CUGAUGAGGCGAAAGGCGGAA	ACUGGGA
2254	UGAUGU	CUGAUGAGGCGAAAGGCGGAA	ACADGGA
2259	GACCUUG	CUGAUGAGGCGAAAGGCGGAA	AUGUGAC
2260	UGACCUU	CUGAUGAGGCGAAAGGCGGAA	AAUGUGA
2266	ACCUGGU	CUGAUGAGGCGAAAGGCGGAA	ACCUUGA
2274	ACAACUG	CUGAUGAGGCGAAAGGCGGAA	ACCUGGU
2279	CCUGUAC	CUGAUGAGGCGAAAGGCGGAA	ACUGUAC
2282	CAACCUG	CUGAUGAGGCGAAAGGCGGAA	ACACUGG
2288	AGUGUAC	CUGAUGAGGCGAAAGGCGGAA	ACCUGUA
2291	UGCAGUG	CUGAUGAGGCGAAAGGCGGAA	ACAACCU
2321	CCCAUUU	CUGAUGAGGCGAAAGGCGGAA	AUCUUUU
2338	CA AUGAG	CUGAUGAGGCGAAAGGCGGAA	AGUCCCA
2339	CCAUGA	CUGAUGAGGCGAAAGGCGGAA	AAGCCCC
2341	GGCCAAU	CUGAUGAGGCGAAAGGCGGAA	AGAAGUC
2344	GUUGGCC	CUGAUGAGGCGAAAGGCGGAA	AUGAGAA
2358	CUGGGGA	CUGAUGAGGCGAAAGGCGGAA	AGGCAGG
2359	UCUGGGG	CUGAUGAGGCGAAAGGCGGAA	AAGGCAG
2360	UUCUGGG	CUGAUGAGGCGAAAGGCGGAA	AAAGGCA
2376	AUAGAAA	CUGAUGAGGCGAAAGGCGGAA	AUCACUC
2377	GAUAGAA	CUGAUGAGGCGAAAGGCGGAA	AAUCACU
2378	CGAUAGA	CUGAUGAGGCGAAAGGCGGAA	AAAUACAC
2379	CGAUAG	CUGAUGAGGCGAAAGGCGGAA	AAAAUCA
2380	GCGAUA	CUGAUGAGGCGAAAGGCGGAA	AAAAAUC
2382	GUGCCGA	CUGAUGAGGCGAAAGGCGGAA	AGAAAAA
2384	UUGUGOC	CUGAUGAGGCGAAAGGCGGAA	AUAGAAA
2399	GUCCAU	CUGAUGAGGCGAAAGGCGGAA	AGUGCUU
2401	CAGUCCA	CUGAUGAGGCGAAAGGCGGAA	AUAGUGC
2411	GAACCAU	CUGAUGAGGCGAAAGGCGGAA	ACCAGUC
2417	ACCUGUG	CUGAUGAGGCGAAAGGCGGAA	ACCAUUA
2418	AACCUGU	CUGAUGAGGCGAAAGGCGGAA	AAACAUU
2425	AUCUCUG	CUGAUGAGGCGAAAGGCGGAA	ACCUGUG
2426	AADCUCU	CUGAUGAGGCGAAAGGCGGAA	AAACUGU
2433	ACUGGGU	CUGAUGAGGCGAAAGGCGGAA	AUCUCUG
2434	CACUGGG	CUGAUGAGGCGAAAGGCGGAA	AADCUCU
2448	GAGGAUU	CUGAUGAGGCGAAAGGCGGAA	AGGCCUC
2449	GGAGGAA	CUGAUGAGGCGAAAGGCGGAA	AAGGOCU
2451	AGGGAGG	CUGAUGAGGCGAAAGGCGGAA	AUAAGGC
2452	AAGGGAG	CUGAUGAGGCGAAAGGCGGAA	AADUAGG
2455	GGGAAGG	CUGAUGAGGCGAAAGGCGGAA	AGGAUAU
2459	UGGGGGG	CUGAUGAGGCGAAAGGCGGAA	AGGGAGG
2460	UUGGGGG	CUGAUGAGGCGAAAGGCGGAA	AAGGGAG
2479	GCUAACA	CUGAUGAGGCGAAAGGCGGAA	AGGUGUC
2480	GGCUAAC	CUGAUGAGGCGAAAGGCGGAA	AAGGUGU
2483	GGUGGCU	CUGAUGAGGCGAAAGGCGGAA	ACAAAGG
2484	AGGUGGC	CUGAUGAGGCGAAAGGCGGAA	AACAAGG
2492	GGGUGGG	CUGAUGAGGCGAAAGGCGGAA	AGGUGGC
2504	AGAAADG	CUGAUGAGGCGAAAGGCGGAA	AUGUGGG
2508	UGGCAGA	CUGAUGAGGCGAAAGGCGGAA	AUGUAUG
2509	CUGGCAG	CUGAUGAGGCGAAAGGCGGAA	AAUGUAU

2510	ACUGGCA	CUGAUGAGGCOGAAAGGCOGAA	AAADGUA
2520	CAUUGUG	CUGAUGAGGCOGAAAGGCOGAA	ACACUGG
2521	UCAUGUG	CUGAUGAGGCOGAAAGGCOGAA	AACACUG
2533	GACCGCU	CUGAUGAGGCOGAAAGGCOGAA	AGUGUCA
2540	CAGACAU	CUGAUGAGGCOGAAAGGCOGAA	ACCGCUG
2545	AUGUCCA	CUGAUGAGGCOGAAAGGCOGAA	ACAUGAC
2568	UUGGGCA	CUGAUGAGGCOGAAAGGCOGAA	AUCCCTU
2579	CAAGGCA	CUGAUGAGGCOGAAAGGCOGAA	AGCUUGG
2585	AGAGGAC	CUGAUGAGGCOGAAAGGCOGAA	AGGCADA
2588	ACAAGAG	CUGAUGAGGCOGAAAGGCOGAA	ACAAGGC
2591	AGGACAA	CUGAUGAGGCOGAAAGGCOGAA	AGGACAA
2593	ACAGGAC	CUGAUGAGGCOGAAAGGCOGAA	AGAGGAC
2596	CAAACAG	CUGAUGAGGCOGAAAGGCOGAA	ACAAGAG
2601	AAADGCA	CUGAUGAGGCOGAAAGGCOGAA	ACAGGAC
2602	GAAAUUC	CUGAUGAGGCOGAAAGGCOGAA	AACAGGA
2607	CCAGUGA	CUGAUGAGGCOGAAAGGCOGAA	AUGCAAA
2608	CCAGUGG	CUGAUGAGGCOGAAAGGCOGAA	AAUGCAA
2609	UCCAGAU	CUGAUGAGGCOGAAAGGCOGAA	AAADGCA
2620	ADAGUGC	CUGAUGAGGCOGAAAGGCOGAA	AGCUCCG
2626	GCUGCAA	CUGAUGAGGCOGAAAGGCOGAA	AGUGCAA
2628	GAGCUGC	CUGAUGAGGCOGAAAGGCOGAA	ADAGUGC
2635	GAAACUG	CUGAUGAGGCOGAAAGGCOGAA	AGCUGCA
2640	UGCAGGA	CUGAUGAGGCOGAAAGGCOGAA	ACUGGAG
2641	CUGCAGG	CUGAUGAGGCOGAAAGGCOGAA	AACUGGA
2642	ACUGCAG	CUGAUGAGGCOGAAAGGCOGAA	AAACUGG
2653	GGACCCU	CUGAUGAGGCOGAAAGGCOGAA	AUCACUG
2659	CUUGCAG	CUGAUGAGGCOGAAAGGCOGAA	ACCCUGA
2689	CCUCCAA	CUGAUGAGGCOGAAAGGCOGAA	ACCUUGG
2691	GUCCUCC	CUGAUGAGGCOGAAAGGCOGAA	AUAUCCU
2700	UGGGAGG	CUGAUGAGGCOGAAAGGCOGAA	AGUCCUC
2704	AAGCUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGAGU
2711	CCUCCCA	CUGAUGAGGCOGAAAGGCOGAA	AGCUGGG
2712	CCCUUCC	CUGAUGAGGCOGAAAGGCOGAA	AAGCUGG
2721	CGCGGAU	CUGAUGAGGCOGAAAGGCOGAA	ACCUUUC
2724	ACACGGG	CUGAUGAGGCOGAAAGGCOGAA	AUGACCC
2744	CUACACA	CUGAUGAGGCOGAAAGGCOGAA	ACACACA
2750	GCUGUGC	CUGAUGAGGCOGAAAGGCOGAA	ACACADA
2759	AGAGCGA	CUGAUGAGGCOGAAAGGCOGAA	AGCUUGU
2761	ACAGAGC	CUGAUGAGGCOGAAAGGCOGAA	AGAGCUU
2765	GGUGACA	CUGAUGAGGCOGAAAGGCOGAA	AGCGAGA
2769	CCUGGGU	CUGAUGAGGCOGAAAGGCOGAA	ACAGAGC
2797	GAACCAU	CUGAUGAGGCOGAAAGGCOGAA	AUUGCAC
2803	UGCAGUG	CUGAUGAGGCOGAAAGGCOGAA	ACCAUGA
2804	CUGCAGU	CUGAUGAGGCOGAAAGGCOGAA	AAUCCUG
2813	AGGUCAA	CUGAUGAGGCOGAAAGGCOGAA	ACUGCAG
2815	AAAGGUC	CUGAUGAGGCOGAAAGGCOGAA	AGACUGC
2821	AGCCCAA	CUGAUGAGGCOGAAAGGCOGAA	AGGUCAA
2822	GAGCCCA	CUGAUGAGGCOGAAAGGCOGAA	AAGGUCA
2823	UGAGCCC	CUGAUGAGGCOGAAAGGCOGAA	AAAGGUC

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2829	ADCACTU	CUGAUGAGGCGGAAAGGCGGAA	AGGCCAA
2837	GUGGGAG	CUGAUGAGGCGGAAAGGCGGAA	AUCACUU
2840	GAGGUGG	CUGAUGAGGCGGAAAGGCGGAA	AGGAUCA
2847	GGAGGCU	CUGAUGAGGCGGAAAGGCGGAA	AGGUGGG
2853	UACUCAG	CUGAUGAGGCGGAAAGGCGGAA	AGGCUGA
2860	UCCAGC	CUGAUGAGGCGGAAAGGCGGAA	ACTCAGG
2872	GUGAGCC	CUGAUGAGGCGGAAAGGCGGAA	AUGGUCC
2877	GUGUUGU	CUGAUGAGGCGGAAAGGCGGAA	AGCCTAU
2899	AAAAACA	CUGAUGAGGCGGAAAGGCGGAA	AUUGGCC
2900	AAAAAUC	CUGAUGAGGCGGAAAGGCGGAA	AUUUGCC
2904	AAAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AUCAAAU
2905	AAAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AUCAA
2906	AAAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAUCAA
2907	AAAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAACA
2908	AAAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAUC
2909	AAAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAU
2910	AAAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2911	AAAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2912	GAAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2913	UGAAAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2914	CUGAAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2915	UCUGAAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2916	CUCUGAA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2917	UCUCUGA	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2918	GUCUCUG	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2919	CGUCUCU	CUGAUGAGGCGGAAAGGCGGAA	AAAAAAA
2931	GUUGCGA	CUGAUGAGGCGGAAAGGCGGAA	ACCCCGU
2933	AUGUUGC	CUGAUGAGGCGGAAAGGCGGAA	AGACCCC
2941	UCUGGGC	CUGAUGAGGCGGAAAGGCGGAA	AUGUUGC
2951	ACAAAGG	CUGAUGAGGCGGAAAGGCGGAA	AGUCUGG
2952	CACAAAG	CUGAUGAGGCGGAAAGGCGGAA	AAGUCUG
2955	UACACA	CUGAUGAGGCGGAAAGGCGGAA	AGGAAGU
2956	CUACAC	CUGAUGAGGCGGAAAGGCGGAA	AAGGAAG
2961	AUUAACT	CUGAUGAGGCGGAAAGGCGGAA	ACACAAA
2962	UAUUAA	CUGAUGAGGCGGAAAGGCGGAA	AACACAA
2965	CUUUAAU	CUGAUGAGGCGGAAAGGCGGAA	ACTAACA
2966	GCUUUUU	CUGAUGAGGCGGAAAGGCGGAA	AACUAA
2969	AAAGCUU	CUGAUGAGGCGGAAAGGCGGAA	AUUAACT
2975	GUUGAGA	CUGAUGAGGCGGAAAGGCGGAA	AGCUUUA
2976	AGUUGAG	CUGAUGAGGCGGAAAGGCGGAA	AAGCUUU
2977	CAGUUGA	CUGAUGAGGCGGAAAGGCGGAA	AAAGCUU
2979	GGCAGUU	CUGAUGAGGCGGAAAGGCGGAA	AGAAAGC

Table 5

Mouse ICAM HH Ribozyme Sequence
nt. Position Ribozyme Sequence

11	CAACGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
23	AGCAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACCACUG
26	AGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAACCA
31	UGGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
34	CGACCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGAGAA
40	AGGCUAC	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGC
48	CCAGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
54	CCAUAC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCA
58	GGAGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGGCAUG
64	CUGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUG
96	GGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
102	CCAGCAG	CUGAUGAGGCCGAAAGGCCGAA	ACUGGCA
108	GGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
115	AGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAACCA
119	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCC
120	GGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
146	GGAAGCG	CUGAUGAGGCCGAAAGGCCGAA	ACCACUG
152	AGGGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAACGA
158	GGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGA
165	GCAAAAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUCUG
168	GGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUU
185	CUGCAGC	CUGAUGAGGCCGAAAGGCCGAA	ACCCACC
209	GCCAGAG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGGC
227	GCAAAAC	CUGAUGAGGCCGAAAGGCCGAA	ACUUCUG
230	GGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAACUU
237	AGUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCACA
248	UUUAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGGUU
253	UCUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGG
263	CAGUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAACCCU
267	UAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCCCU
293	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
319	GGCUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
335	GUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAG
337	CAGUGUG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGAC
338	UCAGCUC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
359	AGCGGAC	CUGAUGAGGCCGAAAGGCCGAA	ACUGCAC
367	CGGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
374	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
375	GGGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUU
378	ACAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
386	AAACGAA	CUGAUGAGGCCGAAAGGCCGAA	ACAAGGU
394	AGAUCGA	CUGAUGAGGCCGAAAGGCCGAA	AGUCCGG
420	CGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
425	CUGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUG

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427	CACUGCU	CUGAUGAGGCCGAAAGGCCGAA	AGAGCUG
450	GCAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
451	CAAAGGA	CUGAUGAGGCCGAAAGGCCGAA	AGGUUUC
456	AGUGGCU	CUGAUGAGGCCGAAAGGCCGAA	AGGUUAA
495	ACACGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
510	CCCCACG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
564	GGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUGAG
592	CCCAUGU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
607	CAUGAGA	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCU
608	GCAUGAG	CUGAUGAGGCCGAAAGGCCGAA	AADUGGC
609	GGCAUGA	CUGAUGAGGCCGAAAGGCCGAA	AAADUGG
611	GCGGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUU
656	CAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCUU
657	UCAGCUC	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
668	GGUGGCC	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUG
677	AGGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUC
684	AGGACCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAA
692	AAGAUUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCG
693	GCAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
696	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
709	UGAGGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCCGC
720	AGCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUA
723	CGGAGCU	CUGAUGAGGCCGAAAGGCCGAA	AAAAGUU
735	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
738	CCAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AGGCCCA
765	GGAAGCG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUG
769	GGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCC
770	UUCACAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAA
785	GGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGCC
786	AGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGGC
792	CUUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
794	AGUCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGCCACG
807	CCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCOCAG
833	GGGUGUC	CUGAUGAGGCCGAAAGGCCGAA	AGCUUUG
846	CAACGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
851	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
863	CCAGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGUGGCU
866	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
867	UCUCCGG	CUGAUGAGGCCGAAAGGCCGAA	AAACGAU
869	CUUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAAGA
881	ACGGGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAU
885	UCACCUC	CUGAUGAGGCCGAAAGGCCGAA	ACCAAGG
933	CCAGAAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUAUG
936	GCACCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGADUA
978	AGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUGGUA
980	AAAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGACUGU
986	AGCUGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGUA
987	GAGCUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUGU
988	GGAGCUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUUG

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1005	UCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGU
1006	UUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCA
1023	CUUCCGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUCCA
1025	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACUUC
1066	UUUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AGAGUGG
1092	GGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCAGU
1093	UUGGCUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCA
1125	UCAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGUUGGG
1163	GCAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGCUUCG
1164	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
1166	AGAGCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCU
1172	GGUUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACAGGA
1200	UGUGGAG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGAG
1201	CUGUCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGC
1203	ACUGGUG	CUGAUGAGGCCGAAAGGCCGAA	AAAAAGU
1227	GCACACG	CUGAUGAGGCCGAAAGGCCGAA	AUGUACC
1228	AGCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AAGCUUC
1233	CUCUCCG	CUGAUGAGGCCGAAAGGCCGAA	AAACGAA
1238	AGGACCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAC
1264	CUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCUUUC
1267	UUCCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCA
1294	GGCUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCU
1295	CUGCCUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUC
1306	CAUUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUGC
1321	UCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUC
1334	UUUAGGA	CUGAUGAGGCCGAAAGGCCGAA	AUGGCUU
1344	CACUCUC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCAU
1351	UAAUUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCA
1353	CACCUUC	CUGAUGAGGCCGAAAGGCCGAA	ACCCACU
1366	AGUUGUA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUUA
1367	AGGUGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGCU
1368	AGAGUGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUAC
1380	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
1388	AGCCACU	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
1398	GUUCUGU	CUGAUGAGGCCGAAAGGCCGAA	ACAGCCA
1402	AGUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AAGCACA
1408	CCUCCCC	CUGAUGAGGCCGAAAGGCCGAA	AUCUGGC
1410	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACUUC
1421	ACAAAAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUGGG
1425	CUCUACC	CUGAUGAGGCCGAAAGGCCGAA	AGGCAGU
1429	CAGGGGC	CUGAUGAGGCCGAAAGGCCGAA	AUAGAGA
1444	UCCUCCU	CUGAUGAGGCCGAAAGGCCGAA	AGCCUUC
1455	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUUCC
1482	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAACU
1484	CADGAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAACAG
1493	GUUCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCACAG
1500	GGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAU
1503	GAUUGAU	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCC
1506	CGGUUAU	CUGAUGAGGCCGAAAGGCCGAA	AACAUAU

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1509	ACACGGU	CUGAUGAGGCGAAAGGCCGAA	AUGGUAG
1518	CGCCUGG	CUGAUGAGGCGAAAGGCCGAA	ACCAUGA
1530	CCAGAAU	CUGAUGAGGCGAAAGGCCGAA	AUUADAG
1533	GGCCAC	CUGAUGAGGCGAAAGGCCGAA	AUGACCA
1551	AGCUGCU	CUGAUGAGGCGAAAGGCCGAA	AGGCCADG
1559	AGGUGGG	CUGAUGAGGCGAAAGGCCGAA	AGGUGCU
1563	GGUUAUA	CUGAUGAGGCGAAAGGCCGAA	ACAUAAG
1565	GCGGUUA	CUGAUGAGGCGAAAGGCCGAA	AAACAUA
1567	UGGCGGU	CUGAUGAGGCGAAAGGCCGAA	AUAACA
1584	AUAUCCU	CUGAUGAGGCGAAAGGCCGAA	AUCUUC
1592	UAACUUG	CUGAUGAGGCGAAAGGCCGAA	AUAUCCU
1599	CCUUCUG	CUGAUGAGGCGAAAGGCCGAA	AACUUGU
1651	GCUCAGG	CUGAUGAGGCGAAAGGCCGAA	AGGUGGG
1661	CAAAGGA	CUGAUGAGGCGAAAGGCCGAA	AGGUUUC
1663	UUCAAAG	CUGAUGAGGCGAAAGGCCGAA	AAAGGUU
1678	CCAGGCU	CUGAUGAGGCGAAAGGCCGAA	AGGUCCU
1680	CCAGAGG	CUGAUGAGGCGAAAGGCCGAA	AGUGGCU
1681	GCCAGAG	CUGAUGAGGCGAAAGGCCGAA	AAGUGGC
1684	ACAGCCA	CUGAUGAGGCGAAAGGCCGAA	AGGAAGU
1690	AGAUGCA	CUGAUGAGGCGAAAGGCCGAA	AGUCCGG
1691	AAGADCG	CUGAUGAGGCGAAAGGCCGAA	AAGUCCG
1696	CCACCCG	CUGAUGAGGCGAAAGGCCGAA	AUGGGCA
1698	CCCCAGG	CUGAUGAGGCGAAAGGCCGAA	AUAUCCG
1737	GCUGGUA	CUGAUGAGGCGAAAGGCCGAA	AGGUCCU
1750	UGAGGUG	CUGAUGAGGCGAAAGGCCGAA	AGCCGCG
1756	GGGCAGG	CUGAUGAGGCGAAAGGCCGAA	AGGCUUC
1787	UGGGGAC	CUGAUGAGGCGAAAGGCCGAA	AUGUCUC
1790	AUUAAGG	CUGAUGAGGCGAAAGGCCGAA	ACAADGC
1793	UCCAGCC	CUGAUGAGGCGAAAGGCCGAA	AGGACCA
1797	UUUADGU	CUGAUGAGGCGAAAGGCCGAA	ACUGGUG
1802	UCUCCAG	CUGAUGAGGCGAAAGGCCGAA	AUCUGGU
1812	GGCCUGA	CUGAUGAGGCGAAAGGCCGAA	AUCCAGU
1813	UGAGGGU	CUGAUGAGGCGAAAGGCCGAA	AAUGCUG
1825	GCAGAGG	CUGAUGAGGCGAAAGGCCGAA	AGCGUGG
1837	GGAGCUA	CUGAUGAGGCGAAAGGCCGAA	AGGCCADG
1845	GGUGGCG	CUGAUGAGGCGAAAGGCCGAA	AGGCUCC
1856	AAGADCG	CUGAUGAGGCGAAAGGCCGAA	AAGUCCG
1861	UACUGGA	CUGAUGAGGCGAAAGGCCGAA	AUCADGU
1865	CUGAGGC	CUGAUGAGGCGAAAGGCCGAA	ACAAGUG
1868	UUUADGU	CUGAUGAGGCGAAAGGCCGAA	ACUGGUG
1877	AGCUGCU	CUGAUGAGGCGAAAGGCCGAA	AGGCCADG
1901	GUCCCUU	CUGAUGAGGCGAAAGGCCGAA	AGUUUUA
1912	ACUGAUC	CUGAUGAGGCGAAAGGCCGAA	ACUAUAD
1922	UAACUUA	CUGAUGAGGCGAAAGGCCGAA	ACAUDCA
1923	GAUAACU	CUGAUGAGGCGAAAGGCCGAA	AGCAUCA
1928	CUGGUAA	CUGAUGAGGCGAAAGGCCGAA	ACUCUAA
1930	AGCUGGU	CUGAUGAGGCGAAAGGCCGAA	AAACUCU
1964	UGGGGAC	CUGAUGAGGCGAAAGGCCGAA	AUGUCUC
1983	UAACUUG	CUGAUGAGGCGAAAGGCCGAA	AUAUCCU

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1996	GGCUCAG	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCT
2005	GGUCGCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCA
2013	UACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2015	CCACCCC	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCA
2020	CUCAAGAA	CUGAUGAGGCCGAAAGGCCGAA	AACCAAC
2039	CCUCUGC	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGC
2040	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
2057	GGAUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGGAGCA
2061	ACAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGUAG
2071	CUGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGUG
2076	UAGCUCU	CUGAUGAGGCCGAAAGGCCGAA	AGGCUAC
2097	CADCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2098	CGGGGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGUGUG
2115	AUCCUCC	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGC
2128	CUCAADA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2130	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
2145	CAUCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2152	AACUCUA	CUGAUGAGGCCGAAAGGCCGAA	AUUAADA
2156	UAUAATA	CUGAUGAGGCCGAAAGGCCGAA	ACAUCAA
2158	AUUAADA	CUGAUGAGGCCGAAAGGCCGAA	AUACAUC
2159	AAUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AADACAU
2160	AAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUACA
2162	CUAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAADA
2163	AAUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AADACAU
2166	AAUAGAG	CUGAUGAGGCCGAAAGGCCGAA	AUGAAGU
2167	AAUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAUACAU
2170	CUAAAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAADA
2171	GGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AACAACU
2173	CUGGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUCUAA
2174	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2175	AGCUGGU	CUGAUGAGGCCGAAAGGCCGAA	AAACUCU
2176	UAGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AAAACUC
2183	CAUAATA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGU
2185	CUCAADA	CUGAUGAGGCCGAAAGGCCGAA	AUAGCUG
2186	ACUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGCU
2187	UACUCAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUAGC
2189	GGUACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAADA
2196	CADCAAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGUUG
2198	AACAUA	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCC
2199	AUAACA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGGC
2200	CUUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGGAAGA
2201	GCCGACA	CUGAUGAGGCCGAAAGGCCGAA	AAAACUU
2205	UCAGGCC	CUGAUGAGGCCGAAAGGCCGAA	ACAATAA
2210	AGCCACT	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
2220	AGAGAAC	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
2224	GGAUUGA	CUGAUGAGGCCGAAAGGCCGAA	ACCUAG
2226	GCGGCCU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCA
2233	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
2242	GGUCCGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCA

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2248	UGGGAUG	CUGAUGAGGCCGAAAGGCOGAA	AUGGAUA
2254	UCAGUGU	CUGAUGAGGCCGAAAGGCOGAA	AADUGGA
2259	CACCGUG	CUGAUGAGGCCGAAAGGCOGAA	ADUGGAU
2260	GCAACGU	CUGAUGAGGCCGAAAGGCOGAA	AAUGUGA
2266	UCCUGGU	CUGAUGAGGCCGAAAGGCOGAA	ACAUUCC
2274	UCUCCAG	CUGAUGAGGCCGAAAGGCOGAA	AUCUGGU
2279	CUUGCAC	CUGAUGAGGCCGAAAGGCOGAA	ACCCUUC
2282	CAGCUCA	CUGAUGAGGCCGAAAGGCOGAA	ACAGCUU
2288	AGGCCAU	CUGAUGAGGCCGAAAGGCOGAA	ACUUAUA
2291	AGCAGAG	CUGAUGAGGCCGAAAGGCOGAA	ACCACUG
2321	CCCAUGU	CUGAUGAGGCCGAAAGGCOGAA	AUCUUUC
2338	CAGGCAG	CUGAUGAGGCCGAAAGGCOGAA	AGUCUCA
2339	CAAAGGA	CUGAUGAGGCCGAAAGGCOGAA	AGGUUUC
2341	AGGCUGG	CUGAUGAGGCCGAAAGGCOGAA	AGAGGUC
2344	GCUGGAA	CUGAUGAGGCCGAAAGGCOGAA	AUCGAAA
2358	CUGCUGA	CUGAUGAGGCCGAAAGGCOGAA	AGCUGGG
2359	UCUGUUC	CUGAUGAGGCCGAAAGGCOGAA	AAAGCAG
2360	UUCAAAG	CUGAUGAGGCCGAAAGGCOGAA	AAAGGUU
2376	UCAGAAG	CUGAUGAGGCCGAAAGGCOGAA	ACCACCU
2377	CCCAGAA	CUGAUGAGGCCGAAAGGCOGAA	AACCAAC
2378	CAGUAGA	CUGAUGAGGCCGAAAGGCOGAA	AAACCCU
2379	CUUAUGA	CUGAUGAGGCCGAAAGGCOGAA	AAAAGCA
2380	GCCGACA	CUGAUGAGGCCGAAAGGCOGAA	AAAACUU
2382	GSGGCAA	CUGAUGAGGCCGAAAGGCOGAA	AGAGAAU
2384	UUGUGUC	CUGAUGAGGCCGAAAGGCOGAA	ACUGGAU
2399	GUCCACA	CUGAUGAGGCCGAAAGGCOGAA	AGUGUUU
2401	CAGCUCA	CUGAUGAGGCCGAAAGGCOGAA	ACAGCUU
2411	GCAUCCU	CUGAUGAGGCCGAAAGGCOGAA	ACCAGUA
2417	ACGUUUG	CUGAUGAGGCCGAAAGGCOGAA	ACCAUUC
2418	GGCCUGA	CUGAUGAGGCCGAAAGGCOGAA	AUCCAGU
2425	AACCCUC	CUGAUGAGGCCGAAAGGCOGAA	ACCCAUG
2426	AAACUCU	CUGAUGAGGCCGAAAGGCOGAA	AAUUAUU
2433	GCUGGUA	CUGAUGAGGCCGAAAGGCOGAA	AACUCUA
2434	AGCUGGU	CUGAUGAGGCCGAAAGGCOGAA	AAACUCU
2448	GGGCAGG	CUGAUGAGGCCGAAAGGCOGAA	AGGCUUC
2449	GGGCAG	CUGAUGAGGCCGAAAGGCOGAA	AAGGCUU
2451	AGGCAGG	CUGAUGAGGCCGAAAGGCOGAA	AACAGGC
2452	GAGGCAG	CUGAUGAGGCCGAAAGGCOGAA	AAACAGG
2455	GGGCAGG	CUGAUGAGGCCGAAAGGCOGAA	AGGCUUC
2459	GGGGGGG	CUGAUGAGGCCGAAAGGCOGAA	AGUGUGG
2460	CGGGGGG	CUGAUGAGGCCGAAAGGCOGAA	AAGUGUG
2479	GCUGGUA	CUGAUGAGGCCGAAAGGCOGAA	AGGUCUC
2480	GGAUAC	CUGAUGAGGCCGAAAGGCOGAA	ACGGUGA
2483	GGUGGCU	CUGAUGAGGCCGAAAGGCOGAA	ACAUUGG
2484	GACUGGU	CUGAUGAGGCCGAAAGGCOGAA	AAAAAAG
2492	AGGUGGG	CUGAUGAGGCCGAAAGGCOGAA	AGGUGCU
2504	ACAAAAG	CUGAUGAGGCCGAAAGGCOGAA	AGGUGGG
2508	UGGGAUG	CUGAUGAGGCCGAAAGGCOGAA	AUGGAUA
2509	CUGGUAA	CUGAUGAGGCCGAAAGGCOGAA	ACUCUAA

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2510	GCUGGUA	CUGAUGAGGCCGAAAGGCCGAA	AACUCUA
2520	CAUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAAAAG
2521	UGAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AADGCUG
2533	GAUACCU	CUGAUGAGGCCGAAAGGCCGAA	AGCAUCA
2540	CACAGCG	CUGAUGAGGCCGAAAGGCCGAA	ACUGCUG
2545	AGGACCA	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAC
2568	UUUGACA	CUGAUGAGGCCGAAAGGCCGAA	ACTUCAC
2579	CAGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AACUUAU
2585	AGAGAAC	CUGAUGAGGCCGAAAGGCCGAA	AUGCCAG
2588	AUUAGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAADGC
2591	AGGAGCA	CUGAUGAGGCCGAAAGGCCGAA	AGAACCA
2593	GCAGAGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAG
2596	CAUUGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAAAAG
2601	AAACGAA	CUGAUGAGGCCGAAAGGCCGAA	ACACGGU
2602	GGGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGA
2607	CCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUCCGAG
2608	CACAGCG	CUGAUGAGGCCGAAAGGCCGAA	ACUGCUG
2609	UCCUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACAUDCC
2620	GCAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
2626	GCUGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUCGAAA
2628	AGGCUAC	CUGAUGAGGCCGAAAGGCCGAA	AGUGUGC
2635	AGGACCG	CUGAUGAGGCCGAAAGGCCGAA	AGCUGAA
2640	GGCAGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGOC
2641	CUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
2642	GAGGCAG	CUGAUGAGGCCGAAAGGCCGAA	AAACAGG
2653	GCAUCCU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGUA
2659	CUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUC
2689	CCUCGGA	CUGAUGAGGCCGAAAGGCCGAA	ACAUDAG
2691	GGCCUUG	CUGAUGAGGCCGAAAGGCCGAA	AGACAUU
2700	GGGCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUUC
2704	AGGCUGG	CUGAUGAGGCCGAAAGGCCGAA	AGAGGUC
2711	CUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCUGGG
2712	CCCUUCC	CUGAUGAGGCCGAAAGGCCGAA	AGACCTC
2721	CUUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCCUUC
2724	GCACACG	CUGAUGAGGCCGAAAGGCCGAA	AUGUACC
2744	CUGCACG	CUGAUGAGGCCGAAAGGCCGAA	ACCCACC
2750	GGUACUC	CUGAUGAGGCCGAAAGGCCGAA	AUAAAUA
2759	AGAUCCA	CUGAUGAGGCCGAAAGGCCGAA	AGUCOOG
2761	GCAGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUCCU
2765	AGCGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAAAA
2769	CCUGUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAGACU
2797	GGACCAU	CUGAUGAGGCCGAAAGGCCGAA	AUUUCAU
2803	CGCCUGG	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGA
2804	CUGCACG	CUGAUGAGGCCGAAAGGCCGAA	ACCCACC
2813	GGGUCAG	CUGAUGAGGCCGAAAGGCCGAA	ACCGGAG
2815	AAAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGACUGU
2821	CCUCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
2822	AAGUCCG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUCC
2823	UGGAGGC	CUGAUGAGGCCGAAAGGCCGAA	AAAGGCA

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2829	AUGAUUA	CUGAUGAGGCCGAAAGGCOOGAA	AGUCCAG
2837	UCAGAG	CUGAUGAGGCCGAAAGGCOOGAA	ACCACCU
2840	CAGGCAG	CUGAUGAGGCCGAAAGGCOOGAA	AGUCUCA
2847	GGUGGCU	CUGAUGAGGCCGAAAGGCOOGAA	ACADUGG
2853	AACAUAA	CUGAUGAGGCCGAAAGGCOOGAA	AGGCOGC
2860	UCACAGU	CUGAUGAGGCCGAAAGGCOOGAA	ACUUGGC
2872	CUUGGCU	CUGAUGAGGCCGAAAGGCOOGAA	AAGGUCC
2877	GUGAUGG	CUGAUGAGGCCGAAAGGCOOGAA	AGCGGAA
2899	AAGAUCG	CUGAUGAGGCCGAAAGGCOOGAA	AAGGCCG
2900	AAAACUC	CUGAUGAGGCCGAAAGGCOOGAA	AAADUAA
2904	AAUAGAG	CUGAUGAGGCCGAAAGGCOOGAA	AUGAAGU
2905	CAADAGA	CUGAUGAGGCCGAAAGGCOOGAA	AAUGAAG
2906	UAADAAA	CUGAUGAGGCCGAAAGGCOOGAA	ACADCAA
2907	AAAUUAA	CUGAUGAGGCCGAAAGGCOOGAA	AAAUACA
2908	AGCAAAA	CUGAUGAGGCCGAAAGGCOOGAA	AAGCUUC
2909	AGAGCAA	CUGAUGAGGCCGAAAGGCOOGAA	AGAAGCU
2910	AAAUUAA	CUGAUGAGGCCGAAAGGCOOGAA	AAAUACA
2911	AAAUUAA	CUGAUGAGGCCGAAAGGCOOGAA	AAAUACA
2912	GACAUUA	CUGAUGAGGCCGAAAGGCOOGAA	AGAACAA
2913	UGACCAG	CUGAUGAGGCCGAAAGGCOOGAA	AGAGAAA
2914	CUUAUGA	CUGAUGAGGCCGAAAGGCOOGAA	AAAAGCA
2915	UCTAAAU	CUGAUGAGGCCGAAAGGCOOGAA	AAUAAAU
2916	CUCCGGA	CUGAUGAGGCCGAAAGGCOOGAA	ACGAUUA
2917	UCUCCGG	CUGAUGAGGCCGAAAGGCOOGAA	AACGAUU
2918	CUCUCCG	CUGAUGAGGCCGAAAGGCOOGAA	AAACGAA
2919	CGACCCU	CUGAUGAGGCCGAAAGGCOOGAA	AUGAGAA
2931	CUUCCGA	CUGAUGAGGCCGAAAGGCOOGAA	ACCUCCA
2933	CCCUUCC	CUGAUGAGGCCGAAAGGCOOGAA	AGACCCU
2941	UGGGGAC	CUGAUGAGGCCGAAAGGCOOGAA	AUGUCUC
2951	GCAGAGG	CUGAUGAGGCCGAAAGGCOOGAA	AGGUGGG
2952	CACAGCG	CUGAUGAGGCCGAAAGGCOOGAA	ACUGCUG
2955	UGACACA	CUGAUGAGGCCGAAAGGCOOGAA	AGUCACU
2956	UGADUC	CUGAUGAGGCCGAAAGGCOOGAA	AAGGAAA
2961	AGGGGCU	CUGAUGAGGCCGAAAGGCOOGAA	ACACAGA
2962	AAUUAUU	CUGAUGAGGCCGAAAGGCOOGAA	AAUACAU
2965	CUUUAUU	CUGAUGAGGCCGAAAGGCOOGAA	AUUCAAA
2966	CCUCUGC	CUGAUGAGGCCGAAAGGCOOGAA	AGCCAGC
2969	AAAACUU	CUGAUGAGGCCGAAAGGCOOGAA	AUUGAUU
2975	GCUGGUA	CUGAUGAGGCCGAAAGGCOOGAA	AACUCUA
2976	AGUAGAG	CUGAUGAGGCCGAAAGGCOOGAA	AACCCUC
2977	CAGCUCA	CUGAUGAGGCCGAAAGGCOOGAA	ACAGCUU
2979	GGCAUUA	CUGAUGAGGCCGAAAGGCOOGAA	AGRAUGA

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Table 6
Human ICAM Hairpin Ribozyme/Substrate Sequences

Position	nt.	Hairpin Ribozyme Sequence	Substrate
70		GGGCGGG AGAA GCUG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	CAGCA GCC CCCGCCCC
86		GGAGUGCG AGAA GCGC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	GGCCU GCC CGCACUCC
343		CCCAUCAG AGAA GUUU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	AAACU GCC CUGAUUGG
635		GGCCUUGG AGAA GCAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	CUUCG GCC CCAAGGGC
653		UUUUCUCA AGAA GCUU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	GAGCU GUU UGAGAACCA
782		AGACUGGG AGAA GCCC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	GGGCU GUU CCCAGUCU
920		CUGCCACAC AGAA GCCG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	CGGCU GAC GUGUGCAG
1301		ACAUUGGA AGAA GCUU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	CAGCA GAC UCCAAUGU
1373		CCCCGAUG AGAA GUGG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	CCACU GCC CAUGGGG
1521		AUACUUGC AGAA GCUA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	UAGCA GCC GCAGUCAU
1594		CUUUGUA AGAA GUUU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	AUNCA GAC UACACACG
2008		ACCCAAUA AGAA GCAA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	UUGCU GCC UAUUGGGU
2034		UUCUGUA AGAA GUGG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	CCACA GAC UUACAGAA
2125		GGUCAGUA AGAA GCAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	CUUCU GUC UACUGACC
2132		GGGUUGGG AGAA GUUG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	CUACU GAC CCCAACCC
2276		ACCUGUAC AGAA GUAC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	GUACA GUU GUACAGGU
2810		AAGGUCAA AGAA GCAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGGUA	CUACA GUC UUGACCUU

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Table 7
Mouse ICAM Hairpin Ribozyme/Substrate Sequences

Position	nt.	Hairpin Ribozyme Sequence	Substrate
76		GGGAUCAC AGAA GUGA ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	UCACC GUTU GUGAUCCC
164		UGAGGAG AGAA GUUC ACCAGAGAAAACACACACUUGUGUGUACAUAUACCUUGUA	GAACU GUTU CUUCCUCA
252		UCAGTCA AGAA GCUU ACCAGAGAAAACACACACUUGUGUGUACAUAUACCUUGUA	AAGCU GUTU UGAGCTUGA
284		GCACACG AGAA GCTUG ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	CAGCA GUC CGCUGUGC
318		AAGCGAC AGAA GCNC ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	GUGCA GUC GUCCGCTU
447		AGAGCTG AGAA GCGG ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	CGCG GAC CCACGCTU
804		UCUCCUG AGAA GCUU ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	AUGCC GAC CCACGAGA
847		UTUACCA AGAA GUGG ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	CCACU GCC UUGGUAGA
913		AGGAUUG AGAA GCUA ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	UAGCG GAC CAGAUCCU
946		AAGUUGA AGAA GUUA ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	UAACA GUC UACACU
1234		CCCAAGCA AGAA GUCU ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	AGACG GAC UGCTUUGG
1275		AUTUCAG AGAA GCTG ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	CAGCA GAC UCTUAAA
1325		UGCCUCC AGAA GCAG ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	CTGCA GAC GGAAGGCA
1350		CCCCGAG AGAA GCAG ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	CTGCU GCC CAUCGGGG
1534		ACUAAGA AGAA GCGA ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	UGGCA GCC UCUUUGU
1851		GUCCACG AGAA GUAG ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	CUACA GCC CGGUGGAC
1880		AGAAUGA AGAA GCGU ACCAGAGAAAACACACAGUUGUGUGUACAUAUACCUUGUA	ACGCU GAC UUCAUUCU

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Table 8
Rat ICAM Halpin Ribozyme/Substrate Sequences

Position	nt.	Halpin Ribozyme Sequence	Substrate
5		AAAGUGCA AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	CUGCU GCC UGCACUUU
59		GGACGAGA AGAA GCAU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	AUGCU GCC UUGUCUCC
84		GGGAUCNC AGAA GCUA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	UUGCC GUU GUUAUCCC
295		GCACAGUG AGAA GCUU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	CAGCA GAC CACUUGCC
329		AAGCCGAG AGAA GCGU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	ACGCA GUC CUCCGCUU
433		UUCCACCA AGAA GCGC ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	GGCUU GCC UGGUGGAA
626		CAUUCUUG AGAA GUGA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	UACU GUU CAAGAAUG
806		UUCCCAGG AGAA GCAU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	AUGCU GAC CCUGGAGA
849		UCCACUGA AGAA GUGG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	CCACU GCC UCAGUGGA
915		AGGUUCUG AGAA GCCA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	UUGCG GAC CAGACCCU
1182		ACCUCCAA AGAA GCAG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	CUGCG GCC UUGGAGGU
1307		AUGUANGA AGAA GCUU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	CAGCA GAC UCUUACAU
1357		UCCUUCUCC AGAA GCGG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	CUGCA GCC GGAAGGCA
1382		UCCCGAUA AGAA GCGG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	CCGCU GCC UAUCCGGA
1858		GCCACCCA AGAA GUAG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	CUACA GCC UGGUGGOC
1887		AGAAAGAA AGAA GCGU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	AGGCU GAC UUCUUCU
2012		GAGUUGGG AGAA GUGU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	ACACU GUC CCCAACUC
2303		AGACTUCCA AGAA GUGG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	CCACA GCC UGGAGUCU
2539		CCUCCAC AGAA GCUU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGGUA	AAGCU GUU GUGGAGG

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Table 9: Rat ICAM HH Ribozyme Target Sequence

nt. Position	HH Target sequence	nt. Position	HH Target Sequence
11	GAUCCAU U CACACUGA	394	GUGGUGU U CUGAACAG
23	GCUGACU C CUUCUCUA	420	GCACCCU C CCAGGGCA
26	GAACUGU C UUCUCUU	425	CCUGGCU U CUGCCAC
31	CCUCUGU C CUGGUUU	427	UCCUGU U AAAAAACA
34	CUGAAGU C AGAUUAC	450	AAGAACU C AUCCUGG
40	CUCAGGU A CAGCCCC	451	GGGUACU C CCCCAGGC
48	GAGAACU C GGGCGGG	456	CUUGGCU C UGCCACCA
54	CCCCGCU C CCUGAGC	495	GCCACCU C ACUGUGUA
58	CCUGGCU U UAGCCCC	510	GUGUGCU C CGUGGGAA
64	CAUUGCU U CAACCGU	564	GAAAUU U CCAACCA
96	CCUCUGU C CUGGUUU	592	GGGAGU C ACCAGGGA
102	CCCCUGU C CUGGUUGC	607	GAGCCAU U UCUAUGC
108	GGACUGU U GGGGAACU	608	AGCCAUU U CUCAGCU
115	UCCUACU U UGUUCCA	609	GCCAUUU C UCAGGCU
119	GACACUGU C CCAACUC	611	CAAUUUU C AUGCUUA
120	GUUGUAGU C CCGGGGC	656	GUCACUGU U CAAGAUG
146	CCAGACU U GGAACUC	657	UCACUGU C AAGAUGU
152	ACCCGCU C CACUCRA	668	GAACUGU C UUCUCU
158	AUUUCUU C ACCAGUCA	677	GCACCCU C CCAGGGCA
165	UGAACAU A CUUCCCC	684	AGGCAGU C CGGACUU
168	GAACUUU C CUGGUUG	692	CCAGACU U GGAACUC
185	GGGUGAU C CGGCGAG	693	CGGACUU C GADUUCC
209	CAGCCCU A AUUGACC	696	GCCUGUU C CUGGUUU
227	GACCAAGU A ACUGUGAA	709	CAGCAUU A CCCCUC
230	CAAGCUGU U GUGGGAGG	720	CUACACU U UCAGCUC
237	CUGAAGU C GACACCC	723	CAACUUU C AGCUCCA
248	GGCCCCU A CCUAGGA	735	CUCCUGU C CUGGUUGC
253	CACUGCU C AGGGGAGG	738	UCCUGCU C GGGUGGA
263	GAGCCAU U UCUAUGC	765	ACUGUGU U UGAGACU
267	GAGCCU C CUGGUUG	769	UCUUGU U CCCCUGA
293	GAAGCUC U CAAGCUGA	770	CUUGUGU C CCUGGAG
319	CGGAGAU C ACAACGA	785	AGGCUGU U UCCUGCU
335	ACUGUGU U UGAGACU	786	GGCCUGU U CCUGGCU
337	UGUGCUA A UGGUCCU	792	CUCCUGU C CUGGUUGC
338	AAGCUCU C AAGCUGAG	794	UCCUGCU C UGAGCUC
359	CAGCAGU C CUGGCUU	807	GCUCAGU A UACUGGA
367	CAUUGCU U CAACCGU	833	CCUGGGU U GGAGACU
374	UAACCCU C ACCACCU	846	CUGACAGU U AUUUAUG
375	AGAAGCU U CCUGGCU	851	GCUCACU U UAGCAGU
378	ACCCACU C ACAGGGUA	863	CAUUGCU U CAACCGU
386	CGUGUGU U UUGAGCU	866	CCAGGCU C CUCGACA

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867	GACCACTU C CCCAOCUA	1421	GGGUACUU C CCCCAGGC
869	CUCUOCUU C UUGOGAAG	1425	ACCCACCTU C CUCUGGCU
881	AADGGCUU C AACCCGGG	1429	ADACUUGU A GOCUCAGG
885	GACCAAGU A ACUGOGAA	1444	AGAAGGCU C AGGAGGAG
933	UGUGUADU C GUUCOCAG	1455	GGGAGUAT C ACCAGGGA
936	GCAGAGAU U UUGUGOCA	1482	AGGGUACU U CCCCAGG
978	UUGAGAAU C UACAACTU	1484	ACUGGCUU U CUCUCUGC
980	GAGAAUCC A CAACUUUU	1493	CCUGGGU U GGAGACUA
986	CUACAACU U UUCAGCUC	1500	CGUGAAAU U AUGGUCAA
987	UACAACCU U UCAGCUC	1503	GAAAADGU U CCAACCAC
988	ACAACTUU U CAGCOCOC	1506	UGGUCAU A AUUGUUGG
1005	UUCGUGAU C GUGGOGUC	1509	GCCACCAU C ACUGUGUA
1006	GUGGAGAU A UCACAGG	1518	GUCCUGGU C GCGGUGU
1023	CCGGAGGU C UCAGAGG	1530	ACCGGGU C AUAUUUGU
1025	GGAGGUCU C AGAAGGGG	1533	CUGAUCAU U GCGGGCUU
1066	CCUACCUU U GUUCCAA	1551	GUGGOCUU C UGUCUGUA
1092	AGAGGGGU C UCAGCAGA	1559	UGGGAAGU C CCUGUUUA
1093	AGGGGAUU C CAGCOCUU	1563	UCCUACCU U UGUUCCCA
1125	CCCCAACU C UUGUUGAU	1565	UUACAACU A UACCGCC
1163	ACGAGCUU U CUUUUGCU	1567	ACACCUAU U ACGGCCAG
1164	CGAGGCUU C UUUUGCUC	1584	AGGAAGAU C AGGAUADA
1166	ACGCUUCU U UUGCUCUG	1592	CAGGAUAD A CAAGUATC
1172	CUUUUGCU C UGOGGCCU	1599	UACAGAUU A CAGAAGGC
1200	AUCCAAUU C ACACOGAA	1651	CCCCGCCU C CCUGAGCC
1201	UUGGGCUU C UCCACAGG	1661	CCGCACUU U GOCUCUGU
1203	GGGCUUCU C CACAGGUC	1663	GAAACAGU C AADGGACA
1227	UUGGAACU C CAUGGCUU	1678	GAGAACCU C GGCUCGGG
1228	GCGGGCUU C GUGAUCGU	1680	GGGCUUCU C CACAGGUC
1233	CCCCUGGU C CUGGOCGC	1681	GGCUGUU U CCUGCUC
1238	UGUGCUAU A UGGUCCUC	1684	CUGCUCGU A GAOCUCUC
1264	GGAAAGAU C AUAAGGGU	1690	CCCCACCU A CAUACAUU
1267	GUCACUGU U CAAGAADG	1691	CCGACCUU U CGAUCUUC
1294	CAGAGAUU U UGUGOCAG	1696	CUCCUGGU C CUGGUCGC
1295	AGAGGGGU C UCAGCAGA	1698	UCAGAUAD A CCUGGAGA
1306	AGCAGACU C UACADGC	1737	GAUCACAU U CAGGGGUC
1321	AACAGAGU C UGGGGAAA	1750	GUCCAUUU A CACCUAUU
1334	GUUUUGCU U CACAGAGC	1756	CCUCUGCU C CUGGUCCU
1344	UUGGUGCU C AGGUADCC	1787	GAGAACCU C GGCUCGGG
1351	UCAGGCCU A AGAGGACU	1790	GACACUGU C CCAACUC
1353	UAGCAGCU C AACAAUGG	1793	ADGGGCCU C ACCUGGAC
1366	AGGGUACU U CCCCAGG	1797	UCCCGUUU U AAAAACCA
1367	GGGUACUU C CCCCAGGC	1802	GUUCAGAU A UACCGGA
1368	GAUGGUGU C CCGCUGGC	1812	AACAGAGU C UGGGGAAA
1380	CUGCCUAD C GGGADGGU	1813	GCGGGCUU C GUGAUCGU
1388	UGGAGACU A ACUGGAUG	1825	GCCACCAU C ACUGUGUA
1398	CUGGCGUU C ACAGGACA	1837	ACCCACCU C ACAGGGUA
1402	CUGGCGUU U GAGAACUG	1845	AGAGGACU C GGAGGGGC
1408	UUCGUGAU C GCGGCGUC	1856	CCCCUAAU C UGAOCUC
1410	CGAACUAD C GAGUGGAC	1861	CAUGGCUU A UADGGUCC

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1865	UADCCGU	A	GACACAAG	2198	GAUGUCU	C	CGAGGUCA
1868	UCACGAGU	C	ADAADAAU	2199	AGACUCU	A	CADGCCAG
1877	ACAGUACU	U	CCCCAGG	2200	GGGUACU	C	CCCCAGGC
1901	CUAAAACU	C	AAGGUACA	2201	GGGUUCU	C	CACAGGUC
1912	GAACAGAU	C	AADGGACA	2205	UUUUGUGU	C	AGCCACUG
1922	AUGUAAGU	U	AUUGCCUA	2210	UGGAGACU	A	ACUGGADG
1923	UGGAGCCU	C	ACCUUUG	2220	GAGAACU	C	GGCCUGGG
1928	GCUCAGAU	A	UACCCGGA	2224	ACAUACAU	U	CCUACCCU
1930	UGGAGACU	A	ACUGGADG	2226	CUGGACU	C	AGGCCACA
1964	AGAGAUU	U	GUGUCAGC	2233	UCADGCU	C	ACAGAACT
1983	GAGAACU	C	GGCCUGGG	2242	ACACAGCU	C	UCAGUAGU
1996	UGGAGACU	C	UUCAAGCU	2248	CUCCUGGU	C	CUUGUCGC
2005	AUGUAAGU	U	AUUGCCUA	2254	AUCCAAU	C	ACACUGAA
2013	CGUGCCU	A	UCGGGADG	2259	GAUCACAU	U	CACGGUGC
2015	CUGCCUAU	C	GGGADGGU	2260	AUCACAU	C	ACGGGCU
2020	UAUUGAGU	A	CCCGUAC	2266	AUCAGAU	A	UACAAGUU
2039	CGGAGGCU	C	ACAAACGA	2274	GAGCAGGU	U	AACAUGUA
2040	CCUGACCU	C	CUGGAGGU	2279	GGAAAGAU	C	AUAAGGCU
2057	CUGGUCCU	C	CAUGGCU	2282	ACAGUUAU	U	UAUUGAGU
2061	GGGUCCAU	U	UACACCUA	2288	GOCCUGGU	C	CUCCADG
2071	AUAUCUGU	A	GCUCACGG	2291	CAGGUAU	A	CAAGUAC
2076	UGUAGCCU	C	AGGCCUAA	2321	GGAAAGAU	C	AUAAGGCU
2097	CCAACCCU	U	GUUGADGU	2338	UUGGGCU	C	UCCACAGG
2098	CCUGACCU	C	CUGGAGGU	2339	GGGUACU	C	CCCCAGGC
2115	UUCGACU	A	GGGUCCUG	2341	GGGUCCU	C	GGGUCCUA
2128	AGUGCCGU	A	CCAUGAUC	2344	CUGCCUGU	A	GACUCCU
2130	GCCUGGUU	C	CUGCCUCU	2358	CCUGCCU	C	CUCCACA
2145	CCAACCCU	U	GUUGADGU	2359	CCAUCU	C	CCACAGAA
2152	UUGAGAU	C	UACAACU	2360	CUUGGUU	C	CCUGGAAG
2156	UGACAGUU	A	UUUAUUGA	2376	GAACUGCU	C	UUCCUCU
2158	UGADGUU	U	UAUAUAU	2377	GACUCCU	U	CUUAUAUA
2159	GAUGUAU	U	AUAUAUUC	2378	GCUGAU	C	UUUACGA
2160	AUGUAUU	A	UUAADUCA	2379	CUGCUCU	C	CUUUGCG
2162	ACAUCUCCU	A	CCUUGGU	2380	UGAUUCCU	U	UCAAGAGU
2163	UAUUUAU	A	AUUCAGAG	2382	AUUUCCU	C	ACGAGUCA
2166	UGADGUU	U	UAUAUAU	2384	UAUCCGU	A	GACACAAG
2167	GAUGUAU	U	AUAUAUUC	2399	UAUAUACU	A	UGUGGACG
2170	GUADUUAU	U	AADUCAGA	2401	UGUGCUAU	A	UGGUCCUC
2171	CAGUUAU	U	AUUGAGUA	2411	CAUUUCCU	C	AUGCUUCA
2173	UGUGCUAU	A	UGGUCCUC	2417	AUCAGGCU	A	UACAAGUU
2174	UCUCUUAU	A	CCCCUGCU	2418	UCAUGCU	C	ACAGAACT
2175	AUUUCUUU	C	ACGAGUCA	2425	UUUAUAU	U	CAGAGUUC
2176	GAAAAGU	U	CCAACCCAC	2426	CCUGGGGU	U	GGAGACUA
2183	UGACAGUU	A	UUUAUUGA	2433	UCAGAGU	C	UGACAGUU
2185	ACAGUUAU	U	UAUUGAGU	2434	CGGAGGCU	C	ACAAACGA
2186	CAGUUAU	U	AUUGAGUA	2448	UGAACAGU	A	CUUCCCCC
2187	AGUUAUU	A	UUGAGUAC	2449	GAAGCCU	C	CUGCCUCG
2189	UUUAUUU	U	GAGUACCC	2451	GGCCUGU	U	CCUGCCUC
2196	CUGACAGU	U	AUUUAUUG	2452	GCCUGUU	C	CUGCCUCU

2455	ACAUUCCU	A	CCUUGGUU	2761	GGGACUUU	C	GAUCUUCC
2459	CCUUGCCU	C	CCCCACAA	2765	CUUUUGCU	C	UGGGCCCU
2460	CCUACCUU	U	GUUCCCAA	2769	UUUCCUAA	U	ACCCUCCG
2479	UUACACCU	A	UUACCGCC	2797	CGUGAAAU	U	AUGGUCAA
2480	GUUGCCGU	U	GUGAUCCC	2803	CUCAUGCU	U	CACAGAAC
2483	ACCUUGGU	U	CCCAADGU	2804	UCAUGCUU	C	ACAGAACT
2484	CCUUUGUU	C	CCAADGUC	2813	GUUCCCAU	C	CGACCCCU
2492	GACCACTU	C	CCCACTUA	2815	CGGACUUU	C	GAUCUUCC
2504	ACCUACAU	A	CAUUCCTA	2821	CCUGACCU	C	CUGGAGGU
2508	ACAUACAU	U	CCUACCUU	2822	UACAACCU	U	UCAGCUCC
2509	CAUACAUU	C	CUAACCUU	2823	CACCUUUU	C	AGCUCCCA
2510	GUCCAUUU	A	CAUUAUUU	2829	UUGGUGCU	C	AGGUADCC
2520	ACCUUGGU	U	CCCAADGU	2837	CACAGGGU	A	CUUCCCCC
2521	CCUUUGUU	C	CCAADGUC	2840	GCAUCCCU	C	CCAGGGCA
2533	ACAGCAUU	U	ACCCUCCA	2847	UUACCCCU	C	ACCCACCU
2540	UUGGUGCU	C	AGGUADCC	2853	UUGGACCU	U	CGGACUAG
2545	AGGCAGCU	C	CGGACUUU	2860	UCUUUGUU	U	CCUUGGAA
2568	CAGAGAUU	U	UGUGCCAG	2872	GGGUGCUU	C	GGGCUCCA
2579	CCUGCACT	U	UGCCUUGG	2877	UGGAGUCU	C	CCAGCAAC
2585	UGGCUUGU	A	GACCUUUC	2899	AGGCAGCU	C	CGGACUUU
2588	UGGCUUGU	C	CCACAGCC	2900	GGCUGACU	U	CCUUCUCU
2591	CUUUUCCU	C	UUGUGAAG	2904	GAACUGCU	C	UUCCUCCU
2593	UCUUAUUU	A	CCCUUGCU	2905	GGCUGACU	U	CCUUCUCU
2596	CUUUGGUU	C	CUUGUCCG	2906	GUUGAUGU	A	UUUAUUAA
2601	UGUGCUAU	A	UGGUCCUC	2907	CUGCCUUU	C	CCCUUGCG
2602	GUUUGGUU	C	GGGUGGUU	2908	UGAUGUAU	U	UAUUUAUU
2607	GUGGGAGU	A	UCACCAGG	2909	GAACUGCU	C	UUCCUCCU
2608	CUUUAGCU	C	CCGUGGGA	2910	ACUUCUUU	C	UCUAUUAC
2609	UGGAGACU	A	ACUGGADG	2911	UUCCUUCU	C	UAUUUACC
2620	UCAGAGUU	C	UGACAGUU	2912	AUGUAUUU	A	UUUAUUCA
2626	CUUUCAGU	A	GUGGUGCU	2913	UGUGUAUU	C	GUUCCACG
2628	UACAACCU	U	UCAGCUCU	2914	GUUUUAUU	U	AUUUCAGA
2635	UCACAGAU	C	CAAUCCAC	2915	UAUUUAUU	A	AUUUCAGG
2640	GUUCAGGU	A	UCCAUCCA	2916	CUUUUCCU	C	UUGUGAAG
2641	CCCCACCU	A	CAUACAUU	2917	CUUCCUUC	U	GUGAAGAC
2642	GUUUGUUU	C	CUUCCUUC	2918	AUUUCCUU	C	ACGAGUCA
2653	CCACAGGU	C	AGGGUGCU	2919	UUUUGUGU	C	AGCCACUG
2659	AGAAGGGU	C	CUGCAAGC	2931	GAUGGUGU	C	CCGUGGCC
2689	ACUAGGGU	C	CUGAGCUU	2933	UGGAGUCU	C	CCAGCAAC
2691	UCAGGCUU	A	AGAGGACU	2941	CAGUACCU	C	CCCCAGGC
2700	AGGGUAUU	U	CCCCAGGG	2951	ACCAUGCU	U	CCUUCGAC
2704	GACCACTU	C	CCCACTUA	2952	CCGACCUU	U	CGAUUUUC
2711	CCUACCUU	U	AGGAAGGU	2955	UGCUUCCU	C	UGACADGG
2712	CCUACCUU	A	GGAAGGUG	2956	CUUUCCUU	U	GAUCCAAU
2721	GGAAGAAU	C	AUACGGGU	2961	UUUUGUGU	C	AGCCACUG
2724	AAGAUCAU	A	CGGGUUUG	2962	UGUGUAUU	C	GUUCCACG
2744	GGGUGGAU	C	CGUGCAGG	2965	CUUUGAAU	C	AUUAAAGU
2750	GUUCCUGU	U	UAAAAACC	2966	UGGAAGCU	C	UUCAAGCU
2759	GACGAACU	A	UUGAGUGG	2969	GAUCCAAU	A	AAGUUUUA

2975	UGGAAGCU C UUCAAGCU
2976	UAAUAGGU C CUCACCG
2977	GAAGCUCU U CAAGCUGA

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Table 10: Rat ICAM HH Ribozyme Sequences

nt. Position	Rat HH Ribozyme Sequence
11	UCAGUGUG CUGAUGAGGCGCGAAAGGCGCGAA AUUGGATC
23	UAGAGAAG CUGAUGAGGCGCGAAAGGCGCGAA AAGUCAGC
26	AAGAGGAA CUGAUGAGGCGCGAAAGGCGCGAA AGCAGUUC
31	AGGACCAG CUGAUGAGGCGCGAAAGGCGCGAA AGCAGAGG
34	GUUAUUCU CUGAUGAGGCGCGAAAGGCGCGAA AGCUUCAG
40	GGGCUUG CUGAUGAGGCGCGAAAGGCGCGAA ACCUGAG
48	CCAGGOC CUGAUGAGGCGCGAAAGGCGCGAA AGGUUCDC
54	GGUCAGG CUGAUGAGGCGCGAAAGGCGCGAA AGGCGGGG
58	GGAGCUA CUGAUGAGGCGCGAAAGGCGCGAA AGGCAOGG
64	ACGGGUUG CUGAUGAGGCGCGAAAGGCGCGAA AGCCAUUG
96	AGGACCAG CUGAUGAGGCGCGAAAGGCGCGAA AGCAGAGG
102	GCGACCAG CUGAUGAGGCGCGAAAGGCGCGAA ACCAGSAG
108	AGUCCOC CUGAUGAGGCGCGAAAGGCGCGAA AGCAGUCC
115	UGGAACA CUGAUGAGGCGCGAAAGGCGCGAA AGGUAGGA
119	GAGUUGG CUGAUGAGGCGCGAAAGGCGCGAA ACAGUGUC
120	GGCCCGG CUGAUGAGGCGCGAAAGGCGCGAA AUCACAAC
146	GGAGUUC CUGAUGAGGCGCGAAAGGCGCGAA AGGUCCGG
152	UUGAGGUG CUGAUGAGGCGCGAAAGGCGCGAA AGCCGGGU
158	UGACUCGU CUGAUGAGGCGCGAAAGGCGCGAA AAAGAAAU
165	GGGGGAAG CUGAUGAGGCGCGAAAGGCGCGAA ACUGUACA
168	CGAGGCAG CUGAUGAGGCGCGAAAGGCGCGAA AAGGCUUC
185	CCUGCAG CUGAUGAGGCGCGAAAGGCGCGAA AUCCACCC
209	GGUCAGAU CUGAUGAGGCGCGAAAGGCGCGAA AGGGGCTG
227	UUCACAGU CUGAUGAGGCGCGAAAGGCGCGAA ACUUGGUC
230	CCUCCAC CUGAUGAGGCGCGAAAGGCGCGAA ACAGCUUG
237	GGGGUGUC CUGAUGAGGCGCGAAAGGCGCGAA AGCUUCAG
248	UCCUAAG CUGAUGAGGCGCGAAAGGCGCGAA AGGGGGCC
253	CCUCCACU CUGAUGAGGCGCGAAAGGCGCGAA AGGCAGUG
263	GCAUGAGA CUGAUGAGGCGCGAAAGGCGCGAA AUUGGCTC
267	CGAGGCAG CUGAUGAGGCGCGAAAGGCGCGAA AAGGCUUC
293	UCAGCUUG CUGAUGAGGCGCGAAAGGCGCGAA AGAGCUUC
319	UUGUUUGU CUGAUGAGGCGCGAAAGGCGCGAA AUCCUCCG
335	AGUUCUCA CUGAUGAGGCGCGAAAGGCGCGAA AGCACAGU
337	GAGGACCA CUGAUGAGGCGCGAAAGGCGCGAA AUAGCACA
338	CUAGCUU CUGAUGAGGCGCGAAAGGCGCGAA AAGAGCUU
359	AAGCCGAG CUGAUGAGGCGCGAAAGGCGCGAA ACUGGGUG
367	ACGGGUUG CUGAUGAGGCGCGAAAGGCGCGAA AGCCAUUG
374	AGGUGGGU CUGAUGAGGCGCGAAAGGCGCGAA AGGGGUAA
375	GAGGCAGG CUGAUGAGGCGCGAAAGGCGCGAA AGGCUUCU
378	UACCCUGU CUGAUGAGGCGCGAAAGGCGCGAA AGGUGGGU
386	AGCUCCAA CUGAUGAGGCGCGAAAGGCGCGAA ACACAGCG

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394	CTGTUCAG	CUGADGAGGCGGAAAGGCGGAA	AGCACCCAC
420	UGGCGUGG	CUGADGAGGCGGAAAGGCGGAA	AGGGGUGC
425	GGUGGCAG	CUGADGAGGCGGAAAGGCGGAA	AGCGGAGG
427	UGGTUUUU	CUGADGAGGCGGAAAGGCGGAA	AACAGGGA
450	CGCAGGAT	CUGADGAGGCGGAAAGGCGGAA	AGGUUCUU
451	GCCUGGGG	CUGADGAGGCGGAAAGGCGGAA	AAGUACCC
456	UGGUGGCA	CUGADGAGGCGGAAAGGCGGAA	AAGCGGAG
495	UACACAGU	CUGADGAGGCGGAAAGGCGGAA	AUGGUGGC
510	UCCCAACG	CUGADGAGGCGGAAAGGCGGAA	AGCAGCAC
564	GUGGUUGG	CUGADGAGGCGGAAAGGCGGAA	ACAUUUUC
592	UCCUGGGU	CUGADGAGGCGGAAAGGCGGAA	AUACUCC
607	GCADGAGA	CUGADGAGGCGGAAAGGCGGAA	AUUGGCUU
608	AGCAUGAG	CUGADGAGGCGGAAAGGCGGAA	AAUUGGCU
609	AAGCAUCA	CUGADGAGGCGGAAAGGCGGAA	AAAUUGGC
611	UGAAGCAU	CUGADGAGGCGGAAAGGCGGAA	AGAADUG
656	CAUUCUUG	CUGADGAGGCGGAAAGGCGGAA	ACAGGAC
657	ACAUUCUU	CUGADGAGGCGGAAAGGCGGAA	AACAGUGA
668	AAGAGGAA	CUGADGAGGCGGAAAGGCGGAA	AGCAGUUC
677	UGGCGUGG	CUGADGAGGCGGAAAGGCGGAA	AGGGGUGC
684	AAAGUCCG	CUGADGAGGCGGAAAGGCGGAA	AGCGGCUU
692	GGAGUCC	CUGADGAGGCGGAAAGGCGGAA	AGGUUGG
693	GGAGAUUC	CUGADGAGGCGGAAAGGCGGAA	AAAGUCCG
696	AGAGGCAG	CUGADGAGGCGGAAAGGCGGAA	AAACAGGC
709	GUGAGGGG	CUGADGAGGCGGAAAGGCGGAA	AAADGCUU
720	GAGCUGAA	CUGADGAGGCGGAAAGGCGGAA	AGUUGUAG
723	UGGGAGCU	CUGADGAGGCGGAAAGGCGGAA	AAAAGUUG
735	GCGACAG	CUGADGAGGCGGAAAGGCGGAA	ACCAGGAG
738	UCCACCCC	CUGADGAGGCGGAAAGGCGGAA	AGGCAGGA
765	AGUUCUCA	CUGADGAGGCGGAAAGGCGGAA	AGCACAGU
769	UCCAGGGG	CUGADGAGGCGGAAAGGCGGAA	ACACAGGA
770	CUUCCAGG	CUGADGAGGCGGAAAGGCGGAA	AACACAGG
785	AGGCAGGA	CUGADGAGGCGGAAAGGCGGAA	ACAGGCUU
786	GAGGCAGG	CUGADGAGGCGGAAAGGCGGAA	AACAGGOC
792	GCGACAG	CUGADGAGGCGGAAAGGCGGAA	ACCAGGAG
794	GAGCUUCA	CUGADGAGGCGGAAAGGCGGAA	AGGCAGGA
807	UCCAGGUA	CUGADGAGGCGGAAAGGCGGAA	AUCUGAGC
833	UAGUCUCC	CUGADGAGGCGGAAAGGCGGAA	ACCCGAGG
846	CAUUAUU	CUGADGAGGCGGAAAGGCGGAA	ACUGUCAG
851	AGCUGCUA	CUGADGAGGCGGAAAGGCGGAA	AGGUGAGC
863	ACGGGUUG	CUGADGAGGCGGAAAGGCGGAA	AGCCAUUG
866	UGUCAGAG	CUGADGAGGCGGAAAGGCGGAA	AAGCAUGG
867	UAGGUGGG	CUGADGAGGCGGAAAGGCGGAA	AGGUGGUC
869	CUUCGCAA	CUGADGAGGCGGAAAGGCGGAA	AGGAAGAG
881	CACGGGUU	CUGADGAGGCGGAAAGGCGGAA	AAGCCAUU
885	UUCACAGU	CUGADGAGGCGGAAAGGCGGAA	ACUUGGUC
933	CUGGGAAC	CUGADGAGGCGGAAAGGCGGAA	AADACACA
936	UGACACAA	CUGADGAGGCGGAAAGGCGGAA	AUCUCUGC
978	AAGUUGUA	CUGADGAGGCGGAAAGGCGGAA	AUUCUCAA
980	AAAAGUUG	CUGADGAGGCGGAAAGGCGGAA	AGAUUCUC

986 GAGCUGAA CUGAUGAGGCGAAAGGCGGAA AGUUGUAG
987 GGAGCUGA CUGAUGAGGCGAAAGGCGGAA AAGUUGUA
988 GGGAGCUG CUGAUGAGGCGAAAGGCGGAA AAAGUUGU
1005 GAGGOCAC CUGAUGAGGCGAAAGGCGGAA AUCACGAA
1006 CCUGGUGA CUGAUGAGGCGAAAGGCGGAA ACCGCCAC
1023 CCUUCUGA CUGAUGAGGCGAAAGGCGGAA ACCUCCGG
1025 CCCCUCU CUGAUGAGGCGAAAGGCGGAA AGACCUCC
1066 UUGGGAAC CUGAUGAGGCGAAAGGCGGAA AAGGUAGG
1092 UCUGCUGA CUGAUGAGGCGAAAGGCGGAA ACCCCUCU
1093 AGGGGCG CUGAUGAGGCGAAAGGCGGAA AUGCCUCU
1125 AUCACAA CUGAUGAGGCGAAAGGCGGAA AGUUGGGG
1163 AGCAAAAG CUGAUGAGGCGAAAGGCGGAA AGCGUCGU
1164 GAGCAAAA CUGAUGAGGCGAAAGGCGGAA AAGCGGCG
1166 CAGAGCAA CUGAUGAGGCGAAAGGCGGAA AGAAGCGU
1172 AGGCCGCA CUGAUGAGGCGAAAGGCGGAA AGCAAAAG
1200 UUCAGGU CUGAUGAGGCGAAAGGCGGAA AAUUGGAA
1201 CCUGGCA CUGAUGAGGCGAAAGGCGGAA AAGGCCAA
1203 GACUUGG CUGAUGAGGCGAAAGGCGGAA AGAAGGCC
1227 AGCACAG CUGAUGAGGCGAAAGGCGGAA AGUUCCAA
1228 ACGAUCAC CUGAUGAGGCGAAAGGCGGAA AAGGCCGC
1233 GCGACAG CUGAUGAGGCGAAAGGCGGAA ACCAGGAG
1238 GAGGACCA CUGAUGAGGCGAAAGGCGGAA AUAAGACA
1264 ACCCGUAT CUGAUGAGGCGAAAGGCGGAA AUUUUCC
1267 CADUCUG CUGAUGAGGCGAAAGGCGGAA ACAGUGAC
1294 CUGACACA CUGAUGAGGCGAAAGGCGGAA AAUCUCGG
1295 UCUGCUGA CUGAUGAGGCGAAAGGCGGAA ACCCCUCU
1306 GCAUGUAA CUGAUGAGGCGAAAGGCGGAA AGUCUGCU
1321 UUUCCCCA CUGAUGAGGCGAAAGGCGGAA ACUCUGUU
1334 GCUUGGG CUGAUGAGGCGAAAGGCGGAA ACGAATAC
1344 GGAATACU CUGAUGAGGCGAAAGGCGGAA AGCAACGA
1351 AGUCCUCU CUGAUGAGGCGAAAGGCGGAA AGGCCUGA
1353 CCAUUGUU CUGAUGAGGCGAAAGGCGGAA AGCUGCUA
1366 CCUGGGGG CUGAUGAGGCGAAAGGCGGAA AGUAACCU
1367 GCUUGGG CUGAUGAGGCGAAAGGCGGAA AAGUAACC
1368 GGCAGCG CUGAUGAGGCGAAAGGCGGAA ACACCAUC
1380 ACCAUCC CUGAUGAGGCGAAAGGCGGAA AUAAGCAG
1388 CADCCAGU CUGAUGAGGCGAAAGGCGGAA AGUCUCCA
1398 UGUCCUGU CUGAUGAGGCGAAAGGCGGAA ACAGCCAG
1402 CAGUUCUC CUGAUGAGGCGAAAGGCGGAA AAGCACAG
1408 GAGGCCAC CUGAUGAGGCGAAAGGCGGAA AUCACGAA
1410 GUCCACUC CUGAUGAGGCGAAAGGCGGAA AUAUUGCG
1421 GCCUGGG CUGAUGAGGCGAAAGGCGGAA AAGUACCC
1425 AGCCAGAG CUGAUGAGGCGAAAGGCGGAA AGGUGGGU
1429 CCUGAGGC CUGAUGAGGCGAAAGGCGGAA ACAAGUAT
1444 CUCCUCCU CUGAUGAGGCGAAAGGCGGAA AGCCUUCU
1455 UCCCUGGU CUGAUGAGGCGAAAGGCGGAA AUACUCCC
1482 CCUGGGGG CUGAUGAGGCGAAAGGCGGAA AGUAACCU
1484 GCAAGAGG CUGAUGAGGCGAAAGGCGGAA AGACAGU
1493 UAGUCUCC CUGAUGAGGCGAAAGGCGGAA ACCCCAGG

1500 UUGACCAU CUGAUGAGGCOGAAAGGCOGAA ADUUCACG
1503 GUGGUUG CUGAUGAGGCOGAAAGGCOGAA ACAUUUUC
1506 CCAACAAU CUGAUGAGGCOGAAAGGCOGAA ADGACCCA
1509 UACACAGU CUGAUGAGGCOGAAAGGCOGAA AUGGUGGC
1518 ACAACGGC CUGAUGAGGCOGAAAGGCOGAA ACCAGGAC
1530 ACAAUUAT CUGAUGAGGCOGAAAGGCOGAA ACCCAGGU
1533 AAGCCCGC CUGAUGAGGCOGAAAGGCOGAA AUGAUCAG
1551 UACGAGCA CUGAUGAGGCOGAAAGGCOGAA AGGGCCAC
1559 UAAACAGG CUGAUGAGGCOGAAAGGCOGAA ACUUCOCA
1563 UGGGAACA CUGAUGAGGCOGAAAGGCOGAA AGGUAGGA
1565 GCGGGUAA CUGAUGAGGCOGAAAGGCOGAA AGGUGUAA
1567 CUGGGGU CUGAUGAGGCOGAAAGGCOGAA ADAGGUGU
1584 UADAUCCU CUGAUGAGGCOGAAAGGCOGAA ADUUCUUU
1592 GUAACUUG CUGAUGAGGCOGAAAGGCOGAA ADAUCCUG
1599 GCUUCUG CUGAUGAGGCOGAAAGGCOGAA AACUUGUA
1651 GGCUCAGG CUGAUGAGGCOGAAAGGCOGAA AGGGGGGG
1661 ACCAGGAC CUGAUGAGGCOGAAAGGCOGAA AAGUGCAG
1663 UGUCCAUU CUGAUGAGGCOGAAAGGCOGAA ADCUGUUC
1678 CCCAGGCC CUGAUGAGGCOGAAAGGCOGAA AGGUUCUC
1680 GACCUUG CUGAUGAGGCOGAAAGGCOGAA AGAAGCCC
1681 GAGGCAGG CUGAUGAGGCOGAAAGGCOGAA AACAGGCC
1684 GAGAGGUC CUGAUGAGGCOGAAAGGCOGAA ACCAGCAG
1690 AADGUADG CUGAUGAGGCOGAAAGGCOGAA AGGUGGGG
1691 GAAGAUUG CUGAUGAGGCOGAAAGGCOGAA AAGUCCGG
1696 GCGACCAU CUGAUGAGGCOGAAAGGCOGAA ACCAGGAG
1698 UCUCCAGG CUGAUGAGGCOGAAAGGCOGAA AUUAUUGA
1737 GCAUCCUG CUGAUGAGGCOGAAAGGCOGAA AUGUGAUC
1750 AAUAGGUG CUGAUGAGGCOGAAAGGCOGAA AAUUGGAC
1756 AGGACCAU CUGAUGAGGCOGAAAGGCOGAA AGCAGAGG
1787 CCCAGGCC CUGAUGAGGCOGAAAGGCOGAA AGGUUCUC
1790 GAGUUGGG CUGAUGAGGCOGAAAGGCOGAA ACAGUGUC
1793 GUCCAGGU CUGAUGAGGCOGAAAGGCOGAA AGGACCAU
1797 UGGUUUUU CUGAUGAGGCOGAAAGGCOGAA AACAGGGA
1802 UCCAGGUA CUGAUGAGGCOGAAAGGCOGAA AUCUGAGC
1812 UUUCUCCA CUGAUGAGGCOGAAAGGCOGAA ACUCUGUU
1813 ACGAUCAC CUGAUGAGGCOGAAAGGCOGAA AAGCCCGC
1825 UACACAGU CUGAUGAGGCOGAAAGGCOGAA AUGGUGGC
1837 UACCCUGU CUGAUGAGGCOGAAAGGCOGAA AGGUGGGU
1845 GCCCCUCC CUGAUGAGGCOGAAAGGCOGAA AGUCCUUC
1856 GCAGGUCA CUGAUGAGGCOGAAAGGCOGAA AUUAGGGG
1861 GGACCAUA CUGAUGAGGCOGAAAGGCOGAA AGCACAUG
1865 CUUGUGUC CUGAUGAGGCOGAAAGGCOGAA ACCGGAUA
1868 AUUUUAUU CUGAUGAGGCOGAAAGGCOGAA ACUGUGUA
1877 CCGGGGGG CUGAUGAGGCOGAAAGGCOGAA AGUACUGU
1901 UGUACCUU CUGAUGAGGCOGAAAGGCOGAA AGUUUUG
1912 UGUCCAUU CUGAUGAGGCOGAAAGGCOGAA AUCUGUUC
1922 UAGGCCAU CUGAUGAGGCOGAAAGGCOGAA ACUUAACU
1923 CUAAAGGU CUGAUGAGGCOGAAAGGCOGAA AGCGUCCA
1928 UCCAGGUA CUGAUGAGGCOGAAAGGCOGAA AUCUGAGC

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1930	CADCCAGU	CUGAUGAGGCOGAAAGGCOOGAA	AGUCUCCA
1964	GCUGACAC	CUGAUGAGGCOGAAAGGCOOGAA	AAAUCCCU
1983	CCCAGGCC	CUGAUGAGGCOGAAAGGCOOGAA	AGGUUCUC
1996	AGCUGGAA	CUGAUGAGGCOGAAAGGCOOGAA	AGCUUCCA
2005	UAGGCAAU	CUGAUGAGGCOGAAAGGCOOGAA	ACUUAACU
2013	CAUCCCGA	CUGAUGAGGCOGAAAGGCOOGAA	AGGCAGCG
2015	ACCAUCCC	CUGAUGAGGCOGAAAGGCOOGAA	AUAGGCAG
2020	GUACAGGG	CUGAUGAGGCOGAAAGGCOOGAA	ACUCAUA
2039	UCGUUUGU	CUGAUGAGGCOGAAAGGCOOGAA	AUCCUCOG
2040	ACCUCCAG	CUGAUGAGGCOGAAAGGCOOGAA	AGGUCAGG
2057	AGCCAUUG	CUGAUGAGGCOGAAAGGCOOGAA	AGGACCAG
2061	UAGGUGUA	CUGAUGAGGCOGAAAGGCOOGAA	AUGGACGC
2071	CCUGAGGC	CUGAUGAGGCOGAAAGGCOOGAA	ACAAGUAU
2076	UUAGGCCU	CUGAUGAGGCOGAAAGGCOOGAA	AGGCUACA
2097	ACAUCAAC	CUGAUGAGGCOGAAAGGCOOGAA	AGAGUUGG
2098	ACCUCCAG	CUGAUGAGGCOGAAAGGCOOGAA	AGGUCAGG
2115	CAGGACCC	CUGAUGAGGCOGAAAGGCOOGAA	AGUCCGAA
2128	GAUCAUGG	CUGAUGAGGCOGAAAGGCOOGAA	ACAGCAAU
2130	AGAGGCAG	CUGAUGAGGCOGAAAGGCOOGAA	AAACAGGC
2145	ACAUCAAC	CUGAUGAGGCOGAAAGGCOOGAA	AGAGUUGG
2152	AAGUUGUA	CUGAUGAGGCOGAAAGGCOOGAA	AUUCUCAA
2156	UCAAUAAA	CUGAUGAGGCOGAAAGGCOOGAA	AACUGUCA
2158	AAUUAUAU	CUGAUGAGGCOGAAAGGCOOGAA	AUAUAUCA
2159	GAUUAUAU	CUGAUGAGGCOGAAAGGCOOGAA	AAUAUAUC
2160	UGAAUUAU	CUGAUGAGGCOGAAAGGCOOGAA	AAUAUAUC
2162	AACRAAGG	CUGAUGAGGCOGAAAGGCOOGAA	AGGAUUGU
2163	CUUGAAAU	CUGAUGAGGCOGAAAGGCOOGAA	AAUAAUAU
2166	AAUUAUAU	CUGAUGAGGCOGAAAGGCOOGAA	AUAUAUCA
2167	GAUUAUAU	CUGAUGAGGCOGAAAGGCOOGAA	AAUAUAUC
2170	UCUGAAAU	CUGAUGAGGCOGAAAGGCOOGAA	AUAUAUAU
2171	UACUCAAU	CUGAUGAGGCOGAAAGGCOOGAA	AAUAUAUC
2173	GAGGACCA	CUGAUGAGGCOGAAAGGCOOGAA	AUAUAUCA
2174	AGCAGGGG	CUGAUGAGGCOGAAAGGCOOGAA	AAUAUAUC
2175	UGACUCGU	CUGAUGAGGCOGAAAGGCOOGAA	AAAGAAAU
2176	GUGGUUGG	CUGAUGAGGCOGAAAGGCOOGAA	ACAUUUUC
2183	UCAAUAAA	CUGAUGAGGCOGAAAGGCOOGAA	AACUGUCA
2185	ACUCAUAU	CUGAUGAGGCOGAAAGGCOOGAA	AUAUAUCA
2186	UACUCAAU	CUGAUGAGGCOGAAAGGCOOGAA	AAUAUAUC
2187	GUACUCAA	CUGAUGAGGCOGAAAGGCOOGAA	AAUAUAUC
2189	GGGUACUC	CUGAUGAGGCOGAAAGGCOOGAA	AUAUAUAU
2196	CAAUAAAU	CUGAUGAGGCOGAAAGGCOOGAA	ACUGUCAG
2198	UGACUUCG	CUGAUGAGGCOGAAAGGCOOGAA	AGCAUAUC
2199	CUGGCAUG	CUGAUGAGGCOGAAAGGCOOGAA	AAGAGUCU
2200	GCCUGGGG	CUGAUGAGGCOGAAAGGCOOGAA	AAGUAACC
2201	GACCUUGU	CUGAUGAGGCOGAAAGGCOOGAA	AGAAACCC
2205	CAGUGGCU	CUGAUGAGGCOGAAAGGCOOGAA	ACACAAAA
2210	CAUCCAGU	CUGAUGAGGCOGAAAGGCOOGAA	AGUCUCCA
2220	CCCAGGCC	CUGAUGAGGCOGAAAGGCOOGAA	AGGUUCUC
2224	AAGGUAGG	CUGAUGAGGCOGAAAGGCOOGAA	AUGUAUGU

2226	UGUGGOCU	CUGAUGAGGCOGAAAGGCCGAA	AGGUCCAG
2233	AGUUCUGU	CUGAUGAGGCOGAAAGGCCGAA	AAGCAUGA
2242	ACUACUGA	CUGAUGAGGCOGAAAGGCCGAA	AGCUGUGU
2248	GCGACCAG	CUGAUGAGGCOGAAAGGCCGAA	ACCAGGAG
2254	UUCAGUGU	CUGAUGAGGCOGAAAGGCCGAA	AADUGGAT
2259	GCAACGUG	CUGAUGAGGCOGAAAGGCCGAA	ADGUGADC
2260	AGCACCGU	CUGAUGAGGCOGAAAGGCCGAA	AADUGGAT
2266	AACUUGUA	CUGAUGAGGCOGAAAGGCCGAA	ADCCUGAT
2274	UACAUGUU	CUGAUGAGGCOGAAAGGCCGAA	ACUUGCUC
2279	ACCCGUAT	CUGAUGAGGCOGAAAGGCCGAA	ADCUUCC
2282	ACUCAUA	CUGAUGAGGCOGAAAGGCCGAA	AUAACUGU
2288	CADUGGAG	CUGAUGAGGCOGAAAGGCCGAA	ACCAGGCG
2291	GUAACUUG	CUGAUGAGGCOGAAAGGCCGAA	AUAUCCUG
2321	ACCCGUAT	CUGAUGAGGCOGAAAGGCCGAA	AUCUUC
2338	CCUGUGGA	CUGAUGAGGCOGAAAGGCCGAA	AAGCCCA
2339	GCCUGGGG	CUGAUGAGGCOGAAAGGCCGAA	AAGUACCC
2341	UGAGCAC	CUGAUGAGGCOGAAAGGCCGAA	ACAGGCC
2344	GAGAGGUC	CUGAUGAGGCOGAAAGGCCGAA	ACGAGCAG
2358	UGUGGGAG	CUGAUGAGGCOGAAAGGCCGAA	AGGCAGGG
2359	UUCUGUGG	CUGAUGAGGCOGAAAGGCCGAA	ADGGAUGG
2360	CUUCCAGG	CUGAUGAGGCOGAAAGGCCGAA	AACACAAG
2376	AAGAGGAA	CUGAUGAGGCOGAAAGGCCGAA	AGCAGUUC
2377	UAADAGAG	CUGAUGAGGCOGAAAGGCCGAA	AGGAAGUC
2378	UCUGAAA	CUGAUGAGGCOGAAAGGCCGAA	AAADUCCG
2379	CGCAAGAG	CUGAUGAGGCOGAAAGGCCGAA	AAGAGCAG
2380	ACUUGUGA	CUGAUGAGGCOGAAAGGCCGAA	AGAAADCA
2382	UGACUUGU	CUGAUGAGGCOGAAAGGCCGAA	AAAGAAAT
2384	CUUGUGUC	CUGAUGAGGCOGAAAGGCCGAA	ACCGGATA
2399	CGUCCACA	CUGAUGAGGCOGAAAGGCCGAA	AGTAUUTA
2401	GAGGACCA	CUGAUGAGGCOGAAAGGCCGAA	AUAGCACA
2411	UGAAGCAU	CUGAUGAGGCOGAAAGGCCGAA	AGAAADUG
2417	AACUUGUA	CUGAUGAGGCOGAAAGGCCGAA	AUCCUGAT
2418	AGUUCUGU	CUGAUGAGGCOGAAAGGCCGAA	AAGCAUGA
2425	GAACUCUG	CUGAUGAGGCOGAAAGGCCGAA	AUAADUAA
2426	UAGUCUCC	CUGAUGAGGCOGAAAGGCCGAA	ACCCACAG
2433	AACUGUCA	CUGAUGAGGCOGAAAGGCCGAA	AACUCUGA
2434	UGUUGUGU	CUGAUGAGGCOGAAAGGCCGAA	AUCCUCCG
2448	GGGGGAAG	CUGAUGAGGCOGAAAGGCCGAA	ACUGUCCA
2449	CGAGGCAG	CUGAUGAGGCOGAAAGGCCGAA	AAGGCUUC
2451	GAGGCAGG	CUGAUGAGGCOGAAAGGCCGAA	AACAGGCC
2452	AGAGGCAG	CUGAUGAGGCOGAAAGGCCGAA	AAACAGGC
2455	AACAAAGG	CUGAUGAGGCOGAAAGGCCGAA	AGGAADGU
2459	UGUGGGAG	CUGAUGAGGCOGAAAGGCCGAA	AGGCAGGG
2460	UUGGGAAC	CUGAUGAGGCOGAAAGGCCGAA	AAGGUAGG
2479	GGCGGUA	CUGAUGAGGCOGAAAGGCCGAA	AGGUGUA
2480	GGGAUCAC	CUGAUGAGGCOGAAAGGCCGAA	ACGGCGAC
2483	ACAUGGGG	CUGAUGAGGCOGAAAGGCCGAA	ACAAAGGU
2484	GACAUGGG	CUGAUGAGGCOGAAAGGCCGAA	AACAAAGG
2492	UAGGGGGG	CUGAUGAGGCOGAAAGGCCGAA	AGGUGGUC

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2504	UAGGAUUG	CUGAUGAGGCOGAAAGGCOGAA	AUGUAAGU
2508	AAGGUAGG	CUGAUGAGGCOGAAAGGCOGAA	AUGUADGU
2509	AAAGGUAG	CUGAUGAGGCOGAAAGGCOGAA	AADGUADG
2510	AADAGGUG	CUGAUGAGGCOGAAAGGCOGAA	AAAUUGAC
2520	ACAUGGGG	CUGAUGAGGCOGAAAGGCOGAA	ACAAAGGU
2521	GACAUUGG	CUGAUGAGGCOGAAAGGCOGAA	AACAAAGG
2533	UGAGGGGU	CUGAUGAGGCOGAAAGGCOGAA	AAUGCOGU
2540	GGAUACCU	CUGAUGAGGCOGAAAGGCOGAA	AGCACCGA
2545	AAAGUCCG	CUGAUGAGGCOGAAAGGCOGAA	AGCUGCCU
2568	CUGACACA	CUGAUGAGGCOGAAAGGCOGAA	AAUCUCUG
2579	CCAGGGCA	CUGAUGAGGCOGAAAGGCOGAA	AGUGCAGG
2585	GAGAGGUC	CUGAUGAGGCOGAAAGGCOGAA	ACGAGCAG
2588	GGCUGUGG	CUGAUGAGGCOGAAAGGCOGAA	AGGAGGCA
2591	CUUCGCAA	CUGAUGAGGCOGAAAGGCOGAA	AGGAAGAG
2593	AGCAGGGG	CUGAUGAGGCOGAAAGGCOGAA	AAUAGAGA
2596	GCGACCAU	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGAG
2601	GAGGACCA	CUGAUGAGGCOGAAAGGCOGAA	AUAGCACCA
2602	ACAACGGC	CUGAUGAGGCOGAAAGGCOGAA	ACCAGGAC
2607	CCUGGUGA	CUGAUGAGGCOGAAAGGCOGAA	ACUCCAC
2608	UCCACCGG	CUGAUGAGGCOGAAAGGCOGAA	AGCUAAAG
2609	CAUCCAGU	CUGAUGAGGCOGAAAGGCOGAA	AGUCUCCA
2620	AAUCUGCA	CUGAUGAGGCOGAAAGGCOGAA	AACUCUGA
2626	AGCAGCAC	CUGAUGAGGCOGAAAGGCOGAA	ACUGAGAG
2628	GGAGCGCA	CUGAUGAGGCOGAAAGGCOGAA	AAGUUGUA
2635	GUGAAUUG	CUGAUGAGGCOGAAAGGCOGAA	AUCUGUGA
2640	UGGAUUGA	CUGAUGAGGCOGAAAGGCOGAA	ACCUAGGC
2641	AADGUADG	CUGAUGAGGCOGAAAGGCOGAA	AGGUGGGG
2642	AGAGGCAG	CUGAUGAGGCOGAAAGGCOGAA	AAACAGGC
2653	AGCAACCU	CUGAUGAGGCOGAAAGGCOGAA	ACCUUGGG
2659	GCUUGCAG	CUGAUGAGGCOGAAAGGCOGAA	ACCCUUCU
2689	AGCUQCAG	CUGAUGAGGCOGAAAGGCOGAA	ACCCUAGU
2691	AGUCCUCU	CUGAUGAGGCOGAAAGGCOGAA	AGGCCUGA
2700	CCUGGGGG	CUGAUGAGGCOGAAAGGCOGAA	AGUAACCU
2704	UAGGUGGG	CUGAUGAGGCOGAAAGGCOGAA	AGGUGGUC
2711	ACCUUCCU	CUGAUGAGGCOGAAAGGCOGAA	AGGUAGGG
2712	CACCUUCC	CUGAUGAGGCOGAAAGGCOGAA	AAGGUAGG
2721	ACCGUUAU	CUGAUGAGGCOGAAAGGCOGAA	AUCUUUCC
2724	CAAACCCG	CUGAUGAGGCOGAAAGGCOGAA	AUGADCUU
2744	CCUGCAGG	CUGAUGAGGCOGAAAGGCOGAA	AUCCACCC
2750	GGUUUUUA	CUGAUGAGGCOGAAAGGCOGAA	ACAGGGAC
2759	CCAUCUGA	CUGAUGAGGCOGAAAGGCOGAA	AGUUUGUC
2761	GGAAGADC	CUGAUGAGGCOGAAAGGCOGAA	AAAGUCCG
2765	AGGCCGCA	CUGAUGAGGCOGAAAGGCOGAA	AGCAAAG
2769	GCAAGGGU	CUGAUGAGGCOGAAAGGCOGAA	AUAGAGAA
2797	UUGACCAU	CUGAUGAGGCOGAAAGGCOGAA	AUUUACCG
2803	GUUCUGUG	CUGAUGAGGCOGAAAGGCOGAA	AGCAUGAG
2804	AGUUCUGU	CUGAUGAGGCOGAAAGGCOGAA	AAGCAUGA
2813	AGGGUCAG	CUGAUGAGGCOGAAAGGCOGAA	AUGGGAGC
2815	GGAAGADC	CUGAUGAGGCOGAAAGGCOGAA	AAAGUCCG

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2821	ACCUCAG	CUGAUGAGGCGAAAGGCGAA	AGGUCAGG
2822	GGAGCUA	CUGAUGAGGCGAAAGGCGAA	AAGUUGUA
2823	UGGGAGCU	CUGAUGAGGCGAAAGGCGAA	AAAAGUUG
2829	GGADACCU	CUGAUGAGGCGAAAGGCGAA	AGCACCGA
2837	GGGGGAAG	CUGAUGAGGCGAAAGGCGAA	ACCCUGGG
2840	UGGCGUGG	CUGAUGAGGCGAAAGGCGAA	AGGGGUGC
2847	AGGUGGGU	CUGAUGAGGCGAAAGGCGAA	AGGGGUA
2853	CUAGUCG	CUGAUGAGGCGAAAGGCGAA	AGADCGAA
2860	UUCGAGG	CUGAUGAGGCGAAAGGCGAA	ACACAAGA
2872	UGAGCAC	CUGAUGAGGCGAAAGGCGAA	ACAGGCC
2877	GGUGCGG	CUGAUGAGGCGAAAGGCGAA	AGACGCC
2899	AAAGUCG	CUGAUGAGGCGAAAGGCGAA	AGCUGCCU
2900	AGAGAAG	CUGAUGAGGCGAAAGGCGAA	AGUCAGCC
2904	AAGAGGA	CUGAUGAGGCGAAAGGCGAA	AGCAGUUC
2905	AGAGAAG	CUGAUGAGGCGAAAGGCGAA	AGUCAGCC
2906	UUAADAA	CUGAUGAGGCGAAAGGCGAA	ACADCRAC
2907	CGCAAGAG	CUGAUGAGGCGAAAGGCGAA	AAGAGCGA
2908	AAUUAUA	CUGAUGAGGCGAAAGGCGAA	ADACADCA
2909	AAGAGGA	CUGAUGAGGCGAAAGGCGAA	AGCAGUUC
2910	GUAADGA	CUGAUGAGGCGAAAGGCGAA	AAGGAAGU
2911	GGUAAUA	CUGAUGAGGCGAAAGGCGAA	AGAAGGAA
2912	UGAADUA	CUGAUGAGGCGAAAGGCGAA	AAATACAU
2913	CUGGAAC	CUGAUGAGGCGAAAGGCGAA	AAACACA
2914	UCGAAAU	CUGAUGAGGCGAAAGGCGAA	ADAAADAC
2915	CUCGAAU	CUGAUGAGGCGAAAGGCGAA	AAUAAUA
2916	CUUGCAA	CUGAUGAGGCGAAAGGCGAA	AGGAAGAG
2917	GUCUCCG	CUGAUGAGGCGAAAGGCGAA	AGAGGAAG
2918	UGACUCU	CUGAUGAGGCGAAAGGCGAA	AAAGAAAU
2919	CAGUGGU	CUGAUGAGGCGAAAGGCGAA	ACACAAA
2931	GGCAGCG	CUGAUGAGGCGAAAGGCGAA	ACACCAUC
2933	GGUGCGG	CUGAUGAGGCGAAAGGCGAA	AGACUCCA
2941	GCCUGGG	CUGAUGAGGCGAAAGGCGAA	AAGUACUG
2951	GUCAGAG	CUGAUGAGGCGAAAGGCGAA	AGCAUGGU
2952	GAAGAUG	CUGAUGAGGCGAAAGGCGAA	AAGUCGG
2955	CCADGUA	CUGAUGAGGCGAAAGGCGAA	AGGAAGCA
2956	AUGAUUC	CUGAUGAGGCGAAAGGCGAA	AAGGAAG
2961	CAGUGGU	CUGAUGAGGCGAAAGGCGAA	ACACRAAA
2962	CUGGAAC	CUGAUGAGGCGAAAGGCGAA	AAACACA
2965	ACUUAUU	CUGAUGAGGCGAAAGGCGAA	AUUCAAAG
2966	AGCUUGA	CUGAUGAGGCGAAAGGCGAA	AGCUUCCA
2969	UAAAACU	CUGAUGAGGCGAAAGGCGAA	AUUGAUUC
2975	AGCUUGA	CUGAUGAGGCGAAAGGCGAA	AGCUUCCA
2976	CAGGUGAG	CUGAUGAGGCGAAAGGCGAA	ACCAUATA
2977	UCAGCUUG	CUGAUGAGGCGAAAGGCGAA	AGAGCUUC

Table 11: Human IL-5 HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	ADGCACU U UCUUUGC	245	AAGAAAU C UUUCAGG
9	UGCACUU U CUUUGCC	247	GAAAUUU U UCAGGGA
10	GCACUUU C UUUGOCA	248	AAAUUUU U CAGGGAA
12	ACUUUCU U UGOCRAA	249	AADUUU C AGGGAAU
13	CUUUCUU U GOCRAAG	257	AGGGAAU A GGCACAC
36	AGAACGU U UCAGAGC	273	GGAGAGU C AAACUGU
37	GAACGUU U CAGAGCC	291	AGGGGGU A CUGUGGA
38	AACGUUU C AGAGCCA	305	AAAGACU A UUCAAAA
56	GGAGGUU U CUGCAUU	307	AGACUUU U CAAAAAC
57	GAUGGUU C UGCAUUU	308	GACUUU C AAAAACT
63	UCUGCAU U UGAGUUU	316	AAAAAAU U GUCCUUA
64	CUGCAUU U GAGUUUG	319	AACUUGU C CUUAAUA
69	UUUGAGU U UGCUAGC	322	UUGUCCU U AAUAAAG
70	UUGAGUU U GCUAGCU	323	UGUCCUU A AUAAAGA
74	GUUUGCU A GCUUUG	326	CCUUAAU A AAGAAAU
78	GCUAGCU C UUGGAGC	334	AAGAAAU A CAUUGAC
80	UAGCUUU U GGAGCUG	338	AAUACAU U GACGGCC
91	GCUUGCU A CGUGUAU	380	GGAGAGU A AACCAAU
97	UACGUGU A UGCCAUC	388	AACCAAU U CCUAGAC
104	ADGCCAU C CCCACAG	389	ACCAAUU C CUAGACU
116	CAGAAAU U CCCACAA	392	AAUCCUU A GACUACC
117	AGAAAUU C CCACAAG	397	CUAGACU A CCUGCAA
130	AGUGCAU U GGUGAAA	409	CAAGAGU U UCUUGGU
145	GAGACCU U GGCACUG	410	AAGAGUU U CUUGGUG
155	CACUGCU U UCUACUC	411	AGAGUUU C UUGGUGU
156	ACUGCUU U CUACUCA	413	AGUUUCU U GGUGUAA
157	CUGCUUU C UACUCAU	419	UUGGUGU A AUAGAACA
159	GCUUUCU A CUCAUUG	437	AGUGGAU A AUAGAAA
162	UUUUAUU C AUUGAAC	440	GGAUAAU A GAAAGUU
165	UACUCAU C GAACUCU	447	AGAAAGU U GAGACUA
171	UGGAACU C UGUGAUU	454	UGAGACU A AACUGGU
179	UGUGAUU A GCCAADG	462	AACUGGU U UGUUGCA
192	UGAGACU C UGAGGAU	463	ACUGGUU U GUUGCAG
200	UGAGGAU U CCUGUUC	466	GGUUUGU U GCAGCCA
201	GAGGAUU C CUGUCC	479	CAAAGAU U UUGGAGG
206	UUCCUGU U CCUGUAC	480	AAAGAUU U UGGAGGA
207	UCCUGUU C CUGUACA	481	AAGAUUU U GGAGGAG
212	UUCCUGU A CAUAAAA	497	AGGACAU U UUAUUGC
216	UGUACAU A AAAAUCA	498	GGACAUU U UACUGCA
222	UAAAAAU C ACCAACT	499	GACAUUU U ACUGCAG

500	ACAUUUU A CUGCAGU	684	UACUUUU U UCUUAUU
531	AAAGAGU C AGGCCUU	685	ACUUUUU U CUUAUUU
538	CAGGCUU U AAUUUUC	686	CUUUUUU C UUAUUUA
539	AGGCCUU A ADUUUCA	688	UUUUUUU U AUUUUAC
542	CCUUAAU U UUCAADA	689	UUUUUUU A UUUAACU
543	CUUAUUU U UCAAUUU	691	UCCUUUU U UAACUUA
544	UUUAUUU U CAUAADA	692	UCUUUUU U AACUUAA
545	UAAUUUU C AAUAUUA	693	CUUAUUU A ACUUUAC
549	UUUCAAU A UAADUUA	697	UUUAACU U AACADUC
551	UCAAUUU A AUUUUAC	698	UUUAACU A ACADUCU
554	ADUAUUU U UAACUUC	703	UUUAACU U CUGUAAA
555	UAUAUUU U AACUUCA	704	UAACUUU C UGUAAAA
556	ADUAUUU A ACUUUCG	708	AUUCUUU A AAADGUC
560	UUUAACU U CAGAGGG	715	AAAUUUU C UGUUAAU
561	UUUAACU C AGAGGGA	719	UGUCUUU U AACUUAA
573	GGAAAGU A AAUAUUU	720	GUUUUUU A ACUUUAU
577	AGUAAAU A UUUCAGG	724	GUUAACU U AAUAUUA
579	UAAAUUU U UCAGGCA	725	UUUAACU A AUUAUUU
580	AAAUUUU U CAGGCAU	728	ACUUUAU A GUUUUUA
581	AAUAUUU C AGGCCUA	731	UAUAUUU A UUUAUGA
588	CAGGCAU A CUGACAC	733	AUAUUUU U UAUGAAA
597	UGACACU U UGCCAGA	734	UAGUUUU U AUGAAAU
598	GACACUU U GCCAGAA	735	AGUUAUU A UGAAAUU
611	AAAGCAU A AAUUUCU	745	AAAUUUU U AAGAUUU
616	ADUAAAU U CUUAAAA	746	AAUUGUU A AGAUUUU
617	UAAAUUU C UUAUAUU	752	UAAGUUU U UGGUAAA
619	AAAUUUU U AAUAUUU	753	AAGAUUU U GGUAAAU
620	AAUUCUU A AAUAADA	757	AUUUGGU A AAUAUUU
625	UUAAAUU A UAUUUCA	761	GGUAAAU U AGUUAUU
627	AAAUUUU A UUUCAGA	762	GUAAAUU A GUUUUUA
629	AAUAUUU U UCAGADA	765	AAUUAUU A UUUAUUU
630	AUAUUUU U CAGAUUU	767	UUAUUUU U UAUUUUA
631	UAUAUUU C AGAUUUC	768	UAGUUUU U AUUUUAU
636	UUCAGAU A UCAGAAU	769	AGUUAUU A UUUAUUG
638	CAGAUUU C AGAUUCA	771	UAUUUUU U UAAGUUU
644	UCAGAAU C AUUGAAG	772	AUUUUUU U AAUGUUA
647	GAUUCUU U GAAGUUA	773	UUUAUUU A AUGUUUU
653	UUGAAGU A UUUUUUU	778	UUUAUUU U AUGUUUU
655	GAAGUUU U UUUUUUU	779	UAAGUUU A UGUUGUG
656	AAGUUUU U UUUUUUA	783	GUUAUUU U GUGUUUU
657	AGUUAUU U CUUUCAG	788	GUUGUUU U CUUAUUA
658	GUUUUUU C CUUUCAG	789	UUUGUUU C UAUAUUA
661	UUUUUUU C CAGGCCA	791	GUGUUUU A AUUAUUA
672	GCAAAAU U GAUAUUA	794	UUCUUAU A AAACAAA
676	AAUUGAU A UACUUUU	805	CAAAAUU A GACACUU
678	UUGAUUU A CUUUUUU		
681	AUAUUUU U UUUUUUU		
682	UAUAUUU U UUUCUUA		

Table 12: Human IL-5 HH Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	GCAAAGA CUGADGAGGCGAAAGGCGCGAA AGUGCAU
9	GGCAAAG CUGADGAGGCGAAAGGCGCGAA AAGUGCA
10	UGGCAAA CUGADGAGGCGAAAGGCGCGAA AAAGUGC
12	UUUGGCA CUGADGAGGCGAAAGGCGCGAA AGAAAGU
13	CUUUGGC CUGADGAGGCGAAAGGCGCGAA AAGAAAG
36	GCUUGA CUGADGAGGCGAAAGGCGCGAA ACCUUCU
37	GGCUCUG CUGADGAGGCGAAAGGCGCGAA AACGUUC
38	UGGCUUC CUGADGAGGCGAAAGGCGCGAA AAACGUU
56	AAUGCAG CUGADGAGGCGAAAGGCGCGAA AGCAUCC
57	AAAUACA CUGADGAGGCGAAAGGCGCGAA AAGCAUC
63	AAACUCA CUGADGAGGCGAAAGGCGCGAA AUGCAGA
64	CAAACUC CUGADGAGGCGAAAGGCGCGAA AAUGCAG
69	GCUAGCA CUGADGAGGCGAAAGGCGCGAA ACUCAAA
70	AGCUAGC CUGADGAGGCGAAAGGCGCGAA AACUCAA
74	CAAGAGC CUGADGAGGCGAAAGGCGCGAA AGCAAAC
78	GCUCCAA CUGADGAGGCGAAAGGCGCGAA AGCUAGC
80	CAGCUCC CUGADGAGGCGAAAGGCGCGAA AGAGCUA
91	AUACACG CUGADGAGGCGAAAGGCGCGAA AGGCAGC
97	GAUGGCA CUGADGAGGCGAAAGGCGCGAA ACACGUA
104	CUGUGGS CUGADGAGGCGAAAGGCGCGAA AUGGCCAU
116	UUGUGGS CUGADGAGGCGAAAGGCGCGAA AUUUCUG
117	CUUGUGG CUGADGAGGCGAAAGGCGCGAA AUUUCU
130	UUUCACC CUGADGAGGCGAAAGGCGCGAA AUGCAU
145	CAGUGCC CUGADGAGGCGAAAGGCGCGAA AGGUCUC
155	GAGUAGA CUGADGAGGCGAAAGGCGCGAA AGCAGUG
156	UGAGUAG CUGADGAGGCGAAAGGCGCGAA AAGCAGU
157	AUGAGUA CUGADGAGGCGAAAGGCGCGAA AAAGCAG
159	CGAUGAG CUGADGAGGCGAAAGGCGCGAA AGAAAGC
162	GUUCGAA CUGADGAGGCGAAAGGCGCGAA AGUAGAA
165	AGAGUUC CUGADGAGGCGAAAGGCGCGAA AUGAGUA
171	AUCAGCA CUGADGAGGCGAAAGGCGCGAA AGUUGCA
179	CADUGGC CUGADGAGGCGAAAGGCGCGAA AUCAGCA
192	ADCCUCA CUGADGAGGCGAAAGGCGCGAA AGUCUCA
200	GAACAGG CUGADGAGGCGAAAGGCGCGAA ADCCUCA
201	GAACAGG CUGADGAGGCGAAAGGCGCGAA AAUCCUC
206	GUACAGG CUGADGAGGCGAAAGGCGCGAA ACAGGAA
207	UGUACAG CUGADGAGGCGAAAGGCGCGAA AACAGGA
212	UUUUAUG CUGADGAGGCGAAAGGCGCGAA ACAGGAA
216	UGAUUUU CUGADGAGGCGAAAGGCGCGAA AUGUACA
222	AGUUGGU CUGADGAGGCGAAAGGCGCGAA AUUUUUA
245	CCUGAAA CUGADGAGGCGAAAGGCGCGAA AUUUCUU

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247	UCCCUGA	CUGAUGAGGCGAAAGGCGGAA	AGAUUUC
248	UCCCUUG	CUGAUGAGGCGAAAGGCGGAA	AAGAUUU
249	AUUCUUU	CUGAUGAGGCGAAAGGCGGAA	AAAGAUU
257	GUGUGCC	CUGAUGAGGCGAAAGGCGGAA	AUUCUUU
273	ACAGUUU	CUGAUGAGGCGAAAGGCGGAA	ACUCUCC
291	UCCACAG	CUGAUGAGGCGAAAGGCGGAA	ACCCUUU
305	UUUUGAA	CUGAUGAGGCGAAAGGCGGAA	AGUCUUU
307	GUUUUUG	CUGAUGAGGCGAAAGGCGGAA	AUAGUCU
308	AGUUUUU	CUGAUGAGGCGAAAGGCGGAA	AAUAGUC
316	UAAGGAC	CUGAUGAGGCGAAAGGCGGAA	AGUUUUU
319	UAUAAG	CUGAUGAGGCGAAAGGCGGAA	ACAAGUU
322	CUUAUU	CUGAUGAGGCGAAAGGCGGAA	AGGACAA
323	UCUUUU	CUGAUGAGGCGAAAGGCGGAA	AAGGACA
326	AUUUUU	CUGAUGAGGCGAAAGGCGGAA	AUUAAGG
334	GUCAADG	CUGAUGAGGCGAAAGGCGGAA	AUUUUUU
338	GGCGGUC	CUGAUGAGGCGAAAGGCGGAA	AUGUAUU
380	AUUGGUU	CUGAUGAGGCGAAAGGCGGAA	ACUCUCC
388	GUCUAGG	CUGAUGAGGCGAAAGGCGGAA	AUUGGUU
389	AGUCUAG	CUGAUGAGGCGAAAGGCGGAA	AAUUGGU
392	GGUAGUC	CUGAUGAGGCGAAAGGCGGAA	AGGAUUU
397	UUGCAGG	CUGAUGAGGCGAAAGGCGGAA	AGUCUAG
409	ACCAAGA	CUGAUGAGGCGAAAGGCGGAA	ACUCUUG
410	CACCAAG	CUGAUGAGGCGAAAGGCGGAA	AAUCUUU
411	ACAACCA	CUGAUGAGGCGAAAGGCGGAA	AAACUUU
413	UUACACC	CUGAUGAGGCGAAAGGCGGAA	AGAAACT
419	UGUUCUU	CUGAUGAGGCGAAAGGCGGAA	ACACCAA
437	UUUCUUU	CUGAUGAGGCGAAAGGCGGAA	AUCCACU
440	AACUUUC	CUGAUGAGGCGAAAGGCGGAA	AUUUUUU
447	UAGUCUC	CUGAUGAGGCGAAAGGCGGAA	ACUUUUU
454	ACCAGUU	CUGAUGAGGCGAAAGGCGGAA	AGUCUCA
462	UGCAACA	CUGAUGAGGCGAAAGGCGGAA	ACCAGUU
463	CUGCAAC	CUGAUGAGGCGAAAGGCGGAA	AACCAGU
466	UGGCUCC	CUGAUGAGGCGAAAGGCGGAA	ACAAACC
479	CCUCCAA	CUGAUGAGGCGAAAGGCGGAA	AUCUUUG
480	UCCUCCA	CUGAUGAGGCGAAAGGCGGAA	AAUCUUU
481	CUUCUCC	CUGAUGAGGCGAAAGGCGGAA	AAAUUUU
497	GCAGUAA	CUGAUGAGGCGAAAGGCGGAA	AUGUCCU
498	UGCAGUA	CUGAUGAGGCGAAAGGCGGAA	AAUGUCC
499	CUGCAGU	CUGAUGAGGCGAAAGGCGGAA	AAADGUC
500	ACUGCAG	CUGAUGAGGCGAAAGGCGGAA	AAAADGU
531	AAGGCUU	CUGAUGAGGCGAAAGGCGGAA	ACUCUUU
538	GAAAAUU	CUGAUGAGGCGAAAGGCGGAA	AGGCCUG
539	UGAAAAU	CUGAUGAGGCGAAAGGCGGAA	AAGGCCU
542	UAUUGAA	CUGAUGAGGCGAAAGGCGGAA	AUUAAGG
543	AUAUUGA	CUGAUGAGGCGAAAGGCGGAA	AAUUAAG
544	UAUAUUG	CUGAUGAGGCGAAAGGCGGAA	AAAUUAA
545	UUUAUUU	CUGAUGAGGCGAAAGGCGGAA	AAAADUA
549	UAAAUUA	CUGAUGAGGCGAAAGGCGGAA	AUUGAAA
551	GUUAAAU	CUGAUGAGGCGAAAGGCGGAA	AUAUUGA

554	GAAGUUA	CUGADGAGGCOGAAAGGCOGAA	AUUAUAU
555	UGAAGUU	CUGADGAGGCOGAAAGGCOGAA	AAUUAUA
556	CUGAAGU	CUGADGAGGCOGAAAGGCOGAA	AAAUUAU
560	CCUCUCG	CUGADGAGGCOGAAAGGCOGAA	AGUUAAA
561	UCCUCUC	CUGADGAGGCOGAAAGGCOGAA	AAGUUAA
573	AAAUUAU	CUGADGAGGCOGAAAGGCOGAA	ACUUUCC
577	CCUGAAA	CUGADGAGGCOGAAAGGCOGAA	AUUUACU
579	UGCCUGA	CUGADGAGGCOGAAAGGCOGAA	AUAUUUA
580	AUGCCUG	CUGADGAGGCOGAAAGGCOGAA	AAUAUUU
581	UADGCCU	CUGADGAGGCOGAAAGGCOGAA	AAAUUAU
588	GUGUCAG	CUGADGAGGCOGAAAGGCOGAA	AUGCCUG
597	UCUGGCA	CUGADGAGGCOGAAAGGCOGAA	AGUGUCA
598	UUCUGGC	CUGADGAGGCOGAAAGGCOGAA	AAGUGUC
611	AGAAUUU	CUGADGAGGCOGAAAGGCOGAA	AUGCUUU
616	UUUUAAG	CUGADGAGGCOGAAAGGCOGAA	AUUUUUU
617	AUUUUAU	CUGADGAGGCOGAAAGGCOGAA	AAUUUUA
619	AAUAUUU	CUGADGAGGCOGAAAGGCOGAA	AGAAUUU
620	UAUAUUU	CUGADGAGGCOGAAAGGCOGAA	AAGAAUU
625	UGAAUAU	CUGADGAGGCOGAAAGGCOGAA	AUUUUAU
627	UCUGAAA	CUGADGAGGCOGAAAGGCOGAA	AUAUUUU
629	UAUCUGA	CUGADGAGGCOGAAAGGCOGAA	AUAUAUU
630	AUAUCUG	CUGADGAGGCOGAAAGGCOGAA	AAUAUAU
631	GAUAUCU	CUGADGAGGCOGAAAGGCOGAA	AAAUUAU
636	AUUCUGA	CUGADGAGGCOGAAAGGCOGAA	AUCUGAA
638	UGAUUCU	CUGADGAGGCOGAAAGGCOGAA	AUAUCUG
644	CUUCAAU	CUGADGAGGCOGAAAGGCOGAA	AUUCUGA
647	AUAUUCU	CUGADGAGGCOGAAAGGCOGAA	AUGAUUC
653	AGGAAAA	CUGADGAGGCOGAAAGGCOGAA	ACTUCAA
655	GGAGGAA	CUGADGAGGCOGAAAGGCOGAA	AUAUUCU
656	UGGAGGA	CUGADGAGGCOGAAAGGCOGAA	AAUAUUC
657	CUGGAGG	CUGADGAGGCOGAAAGGCOGAA	AAAUUAU
658	CCUGGAG	CUGADGAGGCOGAAAGGCOGAA	AAAUUAU
661	UUGCCUG	CUGADGAGGCOGAAAGGCOGAA	AGGAAAA
672	GUUAUUC	CUGADGAGGCOGAAAGGCOGAA	AUUUUGC
676	AAAAGUA	CUGADGAGGCOGAAAGGCOGAA	AUCAUUU
678	AAAAAAG	CUGADGAGGCOGAAAGGCOGAA	AUAUCAA
681	AAGAAAA	CUGADGAGGCOGAAAGGCOGAA	AGUAUAU
682	UAAGAAA	CUGADGAGGCOGAAAGGCOGAA	AAGUAUA
683	AUAAGAA	CUGADGAGGCOGAAAGGCOGAA	AAAGUAU
684	AAUAAGA	CUGADGAGGCOGAAAGGCOGAA	AAAAGUA
685	AAAUUAG	CUGADGAGGCOGAAAGGCOGAA	AAAAAGU
686	UAAAUUA	CUGADGAGGCOGAAAGGCOGAA	AAAAAAG
688	GUUAAAU	CUGADGAGGCOGAAAGGCOGAA	AGAAAAA
689	AGUUAAA	CUGADGAGGCOGAAAGGCOGAA	AAGAAAA
691	UAAGUUA	CUGADGAGGCOGAAAGGCOGAA	AUAAGAA
692	UUAAGUU	CUGADGAGGCOGAAAGGCOGAA	AAUAAGA
693	GUUAAGU	CUGADGAGGCOGAAAGGCOGAA	AAAUUAG
697	GAADGUU	CUGADGAGGCOGAAAGGCOGAA	AGUUAAA
698	AGAADGU	CUGADGAGGCOGAAAGGCOGAA	AAGUUAA

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703	UUUACAG	CUGAUGAGGCCGAAAGGCOGAA	AUGUUAA
704	UUUUACA	CUGAUGAGGCCGAAAGGCOGAA	AAUGUUA
708	GACAUUU	CUGAUGAGGCCGAAAGGCOGAA	ACAGAAU
715	GUUACA	CUGAUGAGGCCGAAAGGCOGAA	ACAUUUU
719	UUUAGUU	CUGAUGAGGCCGAAAGGCOGAA	ACAGACA
720	AUUUAGU	CUGAUGAGGCCGAAAGGCOGAA	AACAGAC
724	UACUUAU	CUGAUGAGGCCGAAAGGCOGAA	AGUUUAC
725	AUACUAU	CUGAUGAGGCCGAAAGGCOGAA	AAGUUAA
728	UAAAUAC	CUGAUGAGGCCGAAAGGCOGAA	AUUUAGU
731	UCAUAAA	CUGAUGAGGCCGAAAGGCOGAA	ACUUAUA
733	UUUCAAU	CUGAUGAGGCCGAAAGGCOGAA	AUACUAU
734	AUUUCAU	CUGAUGAGGCCGAAAGGCOGAA	AAUACUA
735	CAUUUCA	CUGAUGAGGCCGAAAGGCOGAA	AAAUACU
745	AADUCUU	CUGAUGAGGCCGAAAGGCOGAA	ACCAUUU
746	AAAUUCU	CUGAUGAGGCCGAAAGGCOGAA	AACCAUU
752	UUUACCA	CUGAUGAGGCCGAAAGGCOGAA	AUUUUUA
753	AUUUACC	CUGAUGAGGCCGAAAGGCOGAA	AAUUUCU
757	ACUAAUU	CUGAUGAGGCCGAAAGGCOGAA	ACCAAUU
761	AAAUACU	CUGAUGAGGCCGAAAGGCOGAA	AUUUACC
762	UAAAUAC	CUGAUGAGGCCGAAAGGCOGAA	AAUUUAC
765	AAAUAAA	CUGAUGAGGCCGAAAGGCOGAA	ACUUAUU
767	UUAAAUU	CUGAUGAGGCCGAAAGGCOGAA	AUACUAA
768	AUUAAAU	CUGAUGAGGCCGAAAGGCOGAA	AAUACUA
769	CAUUAAA	CUGAUGAGGCCGAAAGGCOGAA	AAAUACU
771	AACAUUA	CUGAUGAGGCCGAAAGGCOGAA	AUAAAUU
772	UAACAUU	CUGAUGAGGCCGAAAGGCOGAA	AAUAAAU
773	AUAACAU	CUGAUGAGGCCGAAAGGCOGAA	AAAUAAA
778	ACAACAU	CUGAUGAGGCCGAAAGGCOGAA	ACAUUAA
779	CACAACA	CUGAUGAGGCCGAAAGGCOGAA	AACAUUA
783	AGAACAC	CUGAUGAGGCCGAAAGGCOGAA	ACAUUAC
788	UUUUUAG	CUGAUGAGGCCGAAAGGCOGAA	ACACAAC
789	UUUAUUA	CUGAUGAGGCCGAAAGGCOGAA	AACACAA
791	GUUUUAU	CUGAUGAGGCCGAAAGGCOGAA	AGAACAC
794	UUUGUUU	CUGAUGAGGCCGAAAGGCOGAA	AUUUAGU
805	AGUUGUC	CUGAUGAGGCCGAAAGGCOGAA	AUUUUUG

Table 13: Mouse IL-5 HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	cGCUUUU c CUUUGCU	253	AGGGGcU A GaCAuAC
11	uCUUcCU U UGCUgAA	259	UagACAU a CUGaAgA
12	CUUcCUU U GUgAAG	269	GaAGAAU C AAAUGU
36	GAagacU U CAGAGuC	269	GaAGAAU c AAaCugU
36	GaAgacU u cAgAGUc	269	GAagaAU c aAAcUgU
37	AAgacUU C AGAGUCA	287	uGGGGGU A CUUGGA
43	UcaGaGU c AUGAgAA	301	AAAUGCU A UUCcAAA
58	GGAUGCU U CUGCacU	301	AAAUGCU a uUCCaaA
59	G AUGCUU C UGCACUU	303	AUGCUAU u CCaAaAc
59	g AUGCUU c uGcAcUU	303	AugCUAU U CcAAAAC
66	CUGCacU U GAGUgUu	304	ugCUAUU C cAAAACc
82	UgAcucU c aGcUGUG	315	AACcUGU C aUUAADA
91	GcUGUGU c uggGCCA	318	cUGUCaU U AAUAAG
112	ugGagAU U CCCAugA	319	UGUCaUU A AUAAAGA
113	gGagAUU C CCAugAG	322	CaUUAUU A AAGAAAU
141	GAGACCU U GaCAcAG	330	AAGAAAU A CAUGGAC
141	GAGACcU U GaCAcAg	334	AAUACAU U GACcGCC
158	gUCcgCU C AcCGAgC	334	AAUaCaU u GACcgCC
167	cCGAgCU C UGUUGAc	384	AggCagU U CCUGGAU
196	UGAGGcU U CCUGUcC	385	gGcagUU C CUgGAUU
197	GAGGcUU C CUGUcCC	393	CUgGAUU A CCUGCAA
197	gAGGCUU c CUGuCCc	405	CAAGAGU U cCUUGGU
202	UUCCUGU c CCUacuc	406	AAGAGUU c CUUGGUG
202	UUCCUGU c CcUAcuc	409	AGUUCcU U GUGUGa
206	UGUCcCU a cuCaUAA	481	UcaCAAU u UAAGUUA
212	UACUCAU a aAAaUCA	482	cAcAAUU U AAGUUaA
212	UacuCAU A AAAAUCA	483	AcAAUUU A AgUUaAa
218	UaaAaaU c aCCAGCU	483	AcAAUUU a aGUUAAa
218	UAAAAAU C ACCAgCU	495	AAAUUGU c AACAgAU
218	uAAAAAU c acCAgCU	553	GCUGUUU c CaUUUAU
232	uaUGCAU U GGAAGAA	557	UUUcCAU U UauUUUU
241	gAGAAAU C UUUCAGG	564	UUauAUU u aUGUCCU
241	gAgAaAU c UUUCAGG	564	UUauAUU u AugUcCU
241	gagAAAU c UUUCAGG	565	uaUAUUU a ugUCCuG
241	gAgAaAU c UUUCAGg	565	UAUAUUU a UGUCcUg
243	gaAAucU U UCAGgGg	569	UUuADGU c cUGUaGU
243	GAAADCU U UCAGGGg	569	uUUADGU c cUGUagU
244	AAAUCCU U CAGGGgc	613	AAAGUGU u uzaCCUU
245	AAUCCUU C AGGGGcU	614	AAgUGuU u aACcUUU

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620	UUAACcU u uDUgUAU	1407	cCagUUU A CUcCAGg
793	caAGgCU u UGUcAU	1407	ccAgUUU a CUCCAGG
816	CUGagUU a UACUCcc	1410	gUUUaCU C CAGGaAA
818	GaguUAU a cUCCcuC	1434	ADgCUUU U aDUUaAU
825	ACUcCcU c CccCUCA	1434	aUgcUUU U AUUUAAu
825	aCUccCU c CccCUcA	1434	aUgcuUU u AUUUAAU
839	AuCCucU U cGUUGCA	1435	UgCUUUU a UuUaAUU
840	uCCucUU c GUUGCAu	1435	ugcUUUU a uUUaAUU
863	cAAgUAU U cCAGGCU	1438	UUUUaCU U AAuUcug
864	AAgUAUU c CAGGCUg	1438	uUUUAUU U AAUUCUg
864	AAGUADU c caggCUg	1439	UUUAUUU A ADUcUgU
913	gAaCUUU u GGuCaG	1443	UUUaAUU c UGuAAaG
917	UcUugyU c CAGauGG	1447	ADUCUGU A AgADGUu
957	UUagcAU c CUUcUc	1458	ugUUcaU a UUAUUUA
960	GCAuccU u UCUcUA	1458	ugUUcAU A uUAUUUA
960	GcaUcCU u uCUcUA	1460	UcAUUAU u AUUUAug
962	ADcUuuU c UCUaGC	1461	UcAUUAU A UUUUUGA
975	gccccUU u AgAUAgA	1463	AUAUAUU U UADGAug
987	aGaUGAU A cuuAAUG	1475	AuGgAUU c aGUAAGU
990	UGAUACU u AAuagcU	1479	AUUcaGU A AgUUAAU
1000	UGACuCU c UugCUGA	1483	aGuAAGU u AAUAUUU
1027	CgggGCU U cCUgCUC	1483	aGUAAgU U AAUAUUU
1034	UCCUGcU C CUaUcuA	1484	GUAAgUU A aUAUUUA
1037	UgcUCCU A UCUAACU	1487	agUUAAU a UUUaUA
1039	cUccuAU c UAACUUC	1487	AgUUAAU A UUUUAUU
1039	cUCCUAU c UAACUUC	1489	UUAAUaU U uAUUAca
1041	CcUAUcU A ACUUCaA	1489	UUAAuAU u UAUUAca
1051	UUcAAuU U AAuaccC	1489	UUAAUAU U UAUUAca
1148	UGAcUUU u cUaUGU	1490	UAUAUUU u AUUAcAc
1213	GCUgGaU u UUGGAaa	1490	UAUAUUU U ADUAcAc
1213	gcUGGAU u uUgGAAA	1490	UAUAUUU U ADUAcAc
1214	cugGAUU U UGGAaaA	1491	AAUAUUU a uuaCAcg
1215	ugGAUUU U GGAaaAG	1491	AAUAUUU a UUAcAcg
1234	gGGACAU c UccuUGC	1491	AaUAUUU A UUAcAcG
1236	GACAUcU c cuUGCAG	1491	AaUAUUU A UUAcAcG
1275	ugGGCCU U AcUUcUC	1494	AUUUAUU a CAcgUAU
1276	gGGCCUU A cUUcUCC	1502	cACGUaU A UaauADu
1280	CUUAcUU c UCcgUgU	1502	cAcgUAU a UAAUAUU
1298	UgAAcUU a AGAAcCA	1507	AUAUAUU a UUCUaaU
1310	gcAAAGU a aAUACcA	1509	ACUAuuU U CUaAUAA
1310	GCAAAgU a aAUAcCa	1509	aUaaUaU U CUAAUAA
1310	GcaAAgU a AAUAccA	1510	UAUAUUU C UaAUAAA
1350	AAAGCAU A AAUggyU	1510	UAUAUUU C UaauAAA
1358	AAADGGU U gyGAugU	1510	UKAUuuU c UaaUAAA
1370	UgUuaUU C AGgUAUC	1510	UaaUaUU C UAAUAAA
1375	UUCAGgU A UCAGggU	1512	aUaUUUU A AUAAAgC
1377	CAGgUAU C AGggUCA	1515	UUCUAUU A AAgCAGa
1383	UCAGggU C AcUGgAG		
1405	cccCAgU U UACUcCA		

Table 14: Human IL-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
86	UPACAGUA AGAA GCUCCA ACCAGAGAAACACACGUGUGUGGUCACAUUAPCCUGGUA	UGGAGCU GGC UACGUGUA
151	GAGUAGAA AGAA GUUCCA ACCAGAGAAACACACGUGUGUGGUCACAUUAPCCUGGUA	UGGAGCU GCU UCUUACUC
172	UGGCUAUC AGAA GAGUUC ACCAGAGAAACACACGUGUGUGGUCACAUUAPCCUGGUA	GAGCUU GCU GAUAGCCA
203	UGUACAGG AGAA GGAUUC ACCAGAGAAACACACGUGUGUGGUCACAUUAPCCUGGUA	GAUUCU GCU CUUUAACA

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Table 16: Mouse IL-5 Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
75	AGCUAGA AGA GACAC ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUU GAC UCUACGU
83	CCAGCAC AGA GAGGU ACCAGGAAACACAGUUGUGUACAUUACUUGUA	ACUUA GCU GUUUGUG
147	GAGGAC AGA GUGUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	UACAC GCU GUUUGUC
150	GAGGAG AGA GUGUG ACCAGGAAACACAGUUGUGUACAUUACUUGUA	CACAGU GUC UGUUAC
154	GUUGUG AGA GACAC ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
168	UGUUG AGA GAGUC ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
199	UGUUG AGA GAGUC ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
274	CCCCAG AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
381	ANUCCAG AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
454	CACUAG AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
499	GUUUG AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
548	UUAUUA AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
701	GCAGAG AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
710	GAGAG AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
870	AGUCAA AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
919	CUUGUC AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
1030	UAGUAG AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
1170	AUGGAC AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
1205	CANAUU AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
1402	CUUGUA AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC
1421	AGCNUC AGA GUUUA ACCAGGAAACACAGUUGUGUACAUUACUUGUA	GUUUG GCU CACUAC

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Position	Hairpin Ribozyme Sequence	Substrate
75	ACCUAGA AGAA GAGAC ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	GUUUU GAC UUCAGCU
83	CCAGCAC AGAA GAGAC ACCAGGAAACACACCGUUGGUCACUUPACUUGUA	ACUUA GCU GUUUGG
147	GAGGAGC AGAA GUUUA ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	UACACA GCU GUUUGG
150	GUAGAGC AGAA GUGUG ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	CAAGCU GUC GGUUAC
154	GUUGGUG AGAA GACAC ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	GUUUG GCU CACAGAC
168	UUCUUC AGAA GAGUC ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	AGUUCU GUU GACACGA
199	UAGUGG AGAA GUAGC ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	GUUUU GUC CUUUGUA
274	CUUUCAG AGAA GUUUA ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	UACACU GUC GUGUGG
381	AAUCCAG AGAA GUUUG ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	CGAGGA GUU CUUGAU
454	CUUUCAG AGAA GUUUG ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	CUAGCU GCU CAGUGG
499	GUUUCAG AGAA GUUUG ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	GUACGA GAU GCAAAAC
548	UAAUUGA AGAA GCAUU ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	AUUGCU GAU UCUUUA
701	CGAGGAG AGAA GAAUU ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	AAUUCU GAU CUUUGC
710	GAGGAGA AGAA GAGAA ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	UUCUUC GAC UUCUUC
870	AGUACAA AGAA GUUUG ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	CAAGCU GCU UUUUUA
919	CUUGUCC AGAA GACUA ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	UGUUA GAU GAGGAG
1030	UAGAUAG AGAA GAAUC ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	GUUUCU GCU CUUUAU
1170	AUGGACA AGAA GAAUC ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	UGAUCA GAC UGUUUA
1205	CAAAUCC AGAA GUUUA ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	UGAGCA GCU GGUUUG
1402	CUAGUA AGAA GGGGA ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	UUUUUA GCU UAUUUG
1421	AACAUAC AGAA GUUUU ACCAGGAAACACAGCGUUGGUCACUUPACUUGUA	AAAUCA GAU GUUUGU

Table 17

Mouse *rel A* HH Target sequence

nt. Position HH Target Sequence nt. Position HH Target Sequence

19	AADGGCU a caCaGgA	467	cCAGGCU c cugUUCg
22	aGCUCcU a cGUGGUG	469	laGCcAU u AGcCAGC
26	CcUCcaU u GcGgACA	473	UuUGAGU C AGauCAG
93	GAuCUGU U uCCCCUC	481	AGCGaAU C CAGACCA
94	luCUGUU u CCCCACA	501	laCCCCU U uCAcGUU
100	UuCCCCU C AUcUUC	502	lCCCCU u CAcGUUC
103	CCUCcAU C UuCCcU	508	UuCAcGU U CUAUAG
105	CUCAUCU U uCCcUCA	509	uCAcGUU C CUAUAGA
106	UCAUCU u CCcUCAG	512	cGUUCU A UAGAgGA
129	CAGGCuU C UGGgCU	514	UGCUAU A GAgGAGC
138	GGgCCU A UGUGGAG	534	GGGACU A uGACuUG
148	UGGAGAU C AUcGAc	556	UGGcCU C UGUUCC
151	AGAUCAU c GAcCAGC	561	CUCUGCU U CCAGGUG
180	AUGCGaU U CCGCUAU	562	UCUGCUU C CAGGUGA
181	UGCGaU C CGCUAA	585	aAgCCAU u AGcCAGc
186	UUCGGCU A uAAaUGC	598	GGCCCCU C CuCCUGA
204	GGGGCU C aGGGGC	613	CcCCUGU C CUcCaC
217	GCAGaU u CCuGGCG	616	CUGUCCU c uCaAUC
239	CACAGAU A CCACCA	617	guCCCUU C CUcAgCC
262	CCACCAU C AAGAUCA	620	CCUCCU C AgCCaug
268	UCRAGAU C AADGGCU	623	UCCUgcU u CCACUC
276	AADGGCU A CACAGGA	628	AUCGyAU u UUGAAuA
301	UuCGaAU C UCCUGG	630	CCgAUuU U UGAuAAc
303	CGaAUCU C CCUGGUC	631	CgAUuU U GAuAAcC
310	CCUGGU C ACCAAGG	638	UGgCcAU u GUauCC
323	GGcCCU C CUcuga	661	CCGAGCU C AAgAUU
326	uCCaCCU C ACCGGCC	667	UCAAGAU C UGGGAG
335	CCGGCCU C AuCCaCA	687	CGgAACT C UGGgAGC
349	AuGAaCU U GUgGGgA	700	GCUGCCU C GGGGGG
352	AGaUcaU c GaAcAGc	715	AUGAGAU C UUCuUgC
375	GAUGGCU a CUADGAG	717	GAGAUU U CuUgCUG
376	AUGGucU C UccGgag	718	AGAUUU C uUgCUGU
378	GGCUaCU A UGAGGCU	721	UuCUCCU c CaaUGcG
391	CUGAcCU C UGCCCAG	751	laGACAU U GAGGUGU
409	GCaGuAU C CAuAGcU	759	GAGGUGU A UUUACG
416	CCgCAGU a UCCauAg	761	GGUGAU U UCAAGGG
417	CAuAGcU U CCAGAAC	762	GUGUAU U CAAGGGA
418	AuAGcU C CAGAACC	763	UGUAUU C ACCGGAC
433	UGGGgAU C CAGUGUG	792	CGAGGCU C CUUUUCu
795	GGCCCCU U UUCuCAA	1167	GAUGAGU U UuCCcCC
796	GCUCUUU U UCuCAAG	1168	AUGAGUU U uCCcCCA
797	CUUCUUU U CuCAAGC	1169	UGAGUUU u CCcCCAU
798	UCCUUU C uCAAGCU	1182	AUGcUGU U aCCaUCA
829	UGGCCAU U GUGUCC	1183	UGcUGUU a CCaUCAg

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834	AUUGUGU U CCGGACu	1184	GGccccU C CUcCOGa
835	UUGUGUU C CGGACuC	1187	GUccCuU c CUcaGCCc
845	GAcuCCU C CgUACGC	1188	UUaCCaU C aGGGCAG
849	CCUCCyU A CGCcGAC	1198	GGgAGuU u AGuCuGa
872	cCAGGCU C CUGUuCG	1209	CAGcCCU a caCCUc
883	UuCGaGU C UCCADGC	1215	cuGGCCU U aGCaCCG
885	CGaGCU C CAUGCAG	1229	GGuCCCU u CCucAGc
905	GCGGCCU U CuGAuCG	1237	CCCAGcU C CUGCCCC
906	CGGCCCU C uGAuCGc	1250	CCAGcCU C CAGgCuC
919	GcGAGCU C AGUGAGC	1268	CCCAGCU C CuGCCcc
936	AUGGAgU U CCAGUAC	1279	CCADUGU c cCuuCcu
937	UGGAgUU C CAGUACu	1281	gUGGgCU C AGCUgcG
942	UUCCAGU A CuUGCCA	1286	AUgAGuU u UccCCCA
953	GCCucAU c CAcAuGA	1309	CuCCUGU u CgAGUCu
962	AGAuGAU C GcCACCG	1315	cCCAGU u CUaCCCC
965	CagUacU u gCCaGAc	1318	CAGUuCU A aCCCCgG
973	ACCGGAU U GAaGAGA	1331	gGGuCCU C CcCAGuC
986	GagACcU u cAAGagu	1334	CuuUuCU C AaGCUGa
996	AGGACcU A UGAGACC	1389	ACGCGU C gGAaGCC
1005	GAGACCU U CAAGAGu	1413	CUGCAGU U UGADGcU
1006	AGACCUU C AAGAGuA	1414	UGCGGUU U GADGcUG
1015	AGAGuAU C AUGAAGA	1437	GGGGCCU U GCUUGGC
1028	GAAGAGU C CUUUCaA	1441	CCUUGCU U GGCACAA
1031	GAGUCCU U UCAauGG	1467	GgaGUGU U CACAGAC
1032	AGUCCUU U CAauGGA	1468	gaGUGUU C ACAGACC
1033	GUCCUUU C AauGGAC	1482	CUGGCAU C uGUgGAC
1058	COGGCCU C CAaCcCG	1486	CuUCgGU a GggAACT
1064	UaCACCU u GAucCAa	1494	GACAACT C aGAGUUU
1072	GyGUuAU U GCUUGGC	1500	UCaGAGU U UCAGCAG
1082	UGUGCCU a CCGaAaA	1501	CaGAGUU U CAGCAGC
1083	aaGOCUU C CCGaAGu	1502	aGAGUUU C AGCAGCU
1092	CGaAaCU C AaCUUCU	1525	gGuGCAU c CCUGUGu
1097	CUCAaCU U CUGUCCC	1566	ADGGAGU A CCGUGAA
1098	UCAaCUU C UGUCCCC	1577	UGAaGCU A UAACTCG
1102	CUUCUGU C CCAAGC	1579	AaGCUAU A ACUCGCC
1125	CAGCCCU A caCCUc	1583	UAUAACU C GCCUgGU
1127	GCCaUAU a gCcUuAC	1588	CUcuCCU A GaGAggG
1131	cAUCCCU c agCaCCA	1622	CCCAGCU C CUGCcCC
1132	AcaCCCU c cCagCAU	1628	UCCUGCU u CggUaGG
1133	UCCaUcU c CagCuUC	1648	CGGGGUU u CCAADG
1137	UUUAcuU u AgCgCgc	1660	cUGaCCU C ugccCAG
1140	cCagCAU C CCUcAGC	1663	cuCUgCU U cCAGGuG
1153	GCACCAU C AACCuUG	1664	uCUgCUU c CAGGuGA
1158	AUCAACU u UGADGAG	1665	CUcgcUU u cGGAGgU
1680	GAAGACU U CCCCCC		
1681	AAGACUU C UCCUCCA		
1683	GACUCCU C CUCCADU		
1686	UUCCCUU C CAUUGCG		
1690	CCUCCAU U GCGGACA		

1704	AUGGACU U CUCUGCU
1705	UGGACUU C UCUGCUC
1707	GACUUCU C UGUCUUA
1721	uuUGAGU C AGAUCAG
1726	GUCAGAU C AGCUCCU
1731	AUCAGCU C CUAAGGU
1734	AGCUCCU A AGGUGCU
1754	CaGugCU C CCaAGAG

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Table 18

Human *rel* A HH Target Sequences

nt. Position HH Target Sequence nt. Position HH Target Sequence

19	AAUGGCU C GUCUGUA	467	GCAGGCU A UCAGUCA
22	GGCUCGU C UGUAGUG	469	AGGCUAU C AGUCAGC
26	CGUCUGU A GUGCAGG	473	UAUCAGU C AGGCGAU
93	GAACUGU U CCCCCUC	481	AGGCGAU C CAGACCA
94	AACUGUU C CCCCCA	501	AACCCCU U CCAAGUU
100	UCCCCU C AUCUUC	502	ACCCCU C CAGUUC
103	CCUCAU C UUCGCG	508	UCCAAGU U CCUAUAG
105	CUCAUCU U CCGGCA	509	CCAAGUU C CUUAAGA
106	UCADCU C CCGGAG	512	AGUUCU A UAGAAGA
129	CAGGCU C UGGCCC	514	UUCCUAU A GAGAGC
138	GGCCCU A UGUGGAG	534	GGGACU A CGACCU
148	UGGAGAU C AUGAGC	556	UGGGCU C UGCUUC
151	AGAUCAU U GAGCAGC	561	CUUGCU U CCAAGG
180	AUGGCU U CGCUAC	562	UCUGCU C CAGGUA
181	UGGCU C CGCUCA	585	GAUCCAU C AGGCGG
186	UUGGCU A CAGUGC	598	GGCCCU C CGCUC
204	GGGCGU C CGGGG	613	CGCGU C CUUCUC
217	GCAGAU C CAGGG	616	UGUUCU U CCUAC
239	CACAGAU A CCACCA	617	UGUCCU C CCACCC
262	CCACAU C AAGUCA	620	CCUUCU C AUCCAU
268	UCAAGAU C AAGGCU	623	UCCUACU C CCAUCU
276	AAGGCU A CACAGGA	628	ACCCAU C UUUGACA
301	UGGCGAU C UCGCG	630	CCCAUCU U UGACAU
303	CGCAUCU C CCGGUC	631	CCAUCU U GACAAC
310	CCUGGU C ACCAAG	638	UGACAU C GUGCCC
323	GGACCU C CUACCG	661	CCGACU C AAGAUU
326	CCUUCU C ACCGGC	667	UCAAGAU C UGCGAG
335	CGGCU C ACCCCA	687	CGAAAU C UGGCAGC
349	ACGAGCU U GUAGGA	700	GCUGCU C GUGGGG
352	AGCUUGU A GGAAAG	715	AUGAGAU C UUCUAC
375	GAUGGU U CUADGAG	717	GAGAUU U CUACUG
376	AUGGCU C UADGAG	718	AGAUU C CUACUG
378	GGCUUCU A UGAGGU	721	UCUUCU A CUGUGU
391	CUGAGCU C UGCGCG	751	AGGACAU U GAGGUG
409	GCUGCAU C CACAGU	759	GAGGUG A UUCACG
416	CCACAGU U UCCAGAA	761	GGUGAUU U UACGGG
417	CACAGU U CCAGAAC	762	GUGUAUU U CACGGGA
418	ACAGUU C CAGAAC	763	UGUAUU C ACCGGAC
433	UGGGAU C CAGUGU	792	CGAGGU C CUUUCG
795	GGCUCCU U UUGCAA	1167	GAGGAGU U UCCACC
796	GCUCUU U UGCAAG	1168	AUGAGUU U CCACCA
797	CUCCUU U CGCAGC	1169	UGAGUU C CCACAU
798	UCCUUU C GCAAGCU	1182	AUGGUGU U UCCUUC
829	UGGCAU U GUGUUC	1183	UGGUGUU U CCUUCG
834	AUUGUGU U CCGACC	1184	GGUGUU C CUUCUG

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835	UUGUGUU C CGGACCC	1187	GUUCCUU U CUGGGCA
845	GACCCUU C CCUACGC	1188	UUUCCUU C UGGGCAG
849	CCUCCUU A CGCAGAC	1198	GGCAGAU C AGCCAGG
872	GCAGGCU C CUGUGCG	1209	CAGGCUU C GGCCUGG
883	UGCGUGU C UCCAUCC	1215	UCCGCUU U GGCCCGG
885	CGUGUCU C CAUCCAG	1229	GGCCCUU C CCAAGU
905	GCGGCUU U CCGACCG	1237	CCAAGU C CUGCCCC
906	CGGCCUU C CGACCGG	1250	CCAGGCU C CAGCCCC
919	GGGAGCU C AGUGAGC	1268	CCUGCUU C CAGCCAU
936	AUGGAUU U CAGUAC	1279	CCAUGGU A UCAGCUC
937	UGGAUUU C CAGUACC	1281	AUGGUUU A AGCUCUG
942	UUCCAGU A CCUGCCA	1286	AUCAGCU C UGGCCCA
953	GCCAGAU A CAGACGA	1309	CCCCUGU C CCAGUCC
962	AGACGCU C GGCACCG	1315	UCCAGU C CUAGCCC
965	CGAUCGU C ACCGGAU	1318	CAGUCCU A GCCCCAG
973	ACCGGAU U GAGGAGA	1331	AGGCCUU C CUCAGGC
986	GAAACGU A AAAGGAC	1334	CCUCCUU C AGGCUGU
996	AGGACAU A UGAGACC	1389	AGCUGU C AGAGGCC
1005	GAGACCU U CAAGAGC	1413	CUGCAGU U UGAGGAU
1006	AGACCUU C AAGAGCA	1414	UGCAGUU U GAUGAGG
1015	AGAGCAU C AUGAAGA	1437	GGGCUU U GCUUGGC
1028	GAAGAGU C CUUUCAG	1441	CCUUGCU U GGCAACA
1031	GAGUCCU U UCAGCGG	1467	GCGUGU U CACAGAC
1032	AGUCCUU U CAGGGGA	1468	CUGUGUU C ACAGACC
1033	GUCCUUU C AGGGGAC	1482	CUGGCAU C CGUCCAC
1058	CCGGCCU C CACCCUG	1486	CAUCCGU C GACAACU
1064	UCCACCU C GAGCCAU	1494	GACAACU C CGAGUUU
1072	GAGCCAU U GCUUGGC	1500	UCCAGU U UCAGCAG
1082	UGUGCCU U CCGCAG	1501	CCAGUU U CAGCAGC
1083	GUGCCUU C CCGCAGC	1502	CGAGUUU C AGCAGCU
1092	CGCAGCU C AGCUUCU	1525	AGGGCAU A CCUGUGG
1097	CUCAGCU U CUGUCCC	1566	AUGGAGU A CCGUGAG
1098	UCAGCUU C UGUCCCC	1577	UGAGGCU A UACUCG
1102	CUUCUGU C CCAAGC	1579	AGGCUAU A ACUCGCC
1125	CAGCCUU A UCCCUUU	1583	UAUAACU C GCUAGU
1127	GCCCUAU C CCUQUAC	1588	CUUGCUU A GUGACAG
1131	UAUCCUU U UAGGUA	1622	CCAGCUU C CUGCUCC
1132	AUCCCUU U ACGUCAU	1628	UCCUGCU C CACUGGG
1133	UCCCUUU A CGUCAUC	1648	CGGGCUU C CCAADUG
1137	UUUACGU C AUCCUG	1660	AUGGCUU C CUUUCAG
1140	ACGUCAU C CCGAGC	1663	GCCUCCU U UCAGGAG
1153	GCAOCAU C AACUAUG	1664	CCUCCUU U CAGGAGA
1158	AUCAACU A UGAGGAG	1665	CUCCUUU C AGGAGAU
1680	GAAGACU U CUCCUCC		
1681	AAGACUU C UCCUCCA		
1683	GACUUCU C CUCCAUU		
1686	UUCUCCU C CAUUGCG		
1690	CCUCCAU U GCGGACA		
1704	ADGGACU U CUCAGCC		

1705	UGGAGUU C UCAGCCC
1707	GACUUCU C AGCCCCG
1721	GCUGAGU C AGAUCAG
1726	GUCAGAU C AGCUCCU
1731	AUCAGCU C CUAAGGG
1734	AGCUCCU A AGGGGGU
1754	CUGCCCC C CCCAGAG

Table 19
 Mouse *rel A* HH Ribozyme Sequences
 nt HH Ribozyme Sequence
 Sequence

19	UCCUGUG	CUGAUGAGGCGGAAAGGCCGAA	AGCCADU
22	CACCAAG	CUGAUGAGGCGGAAAGGCCGAA	AGGAGCU
26	UGUCCGC	CUGAUGAGGCGGAAAGGCCGAA	ADGGAGG
93	GAGGGGA	CUGAUGAGGCGGAAAGGCCGAA	ACAGAUU
94	UGAGGGG	CUGAUGAGGCGGAAAGGCCGAA	AACAGAU
100	GAAAGAU	CUGAUGAGGCGGAAAGGCCGAA	AGGGGAA
103	AGGGAAA	CUGAUGAGGCGGAAAGGCCGAA	ADGAGGS
105	UGAGGGA	CUGAUGAGGCGGAAAGGCCGAA	AGAUGAG
106	CUGAGGG	CUGAUGAGGCGGAAAGGCCGAA	AAGADGA
129	AGGCCCA	CUGAUGAGGCGGAAAGGCCGAA	AAGCCUG
138	CUCCACA	CUGAUGAGGCGGAAAGGCCGAA	AAGGCCC
148	GUUCGAU	CUGAUGAGGCGGAAAGGCCGAA	ADCUCCA
151	GCUGUUC	CUGAUGAGGCGGAAAGGCCGAA	ADGAUCU
180	AUAGCGG	CUGAUGAGGCGGAAAGGCCGAA	ADCGCAU
181	UAUAGCG	CUGAUGAGGCGGAAAGGCCGAA	AADCGCA
186	GCAUUUA	CUGAUGAGGCGGAAAGGCCGAA	AGCGGAA
204	GCCCCCU	CUGAUGAGGCGGAAAGGCCGAA	AGCGGCC
217	CGCCAGG	CUGAUGAGGCGGAAAGGCCGAA	AUAUCUG
239	UUGGUGG	CUGAUGAGGCGGAAAGGCCGAA	ADCUUGG
262	UGAUUUU	CUGAUGAGGCGGAAAGGCCGAA	ADGGUGG
268	AGCCADU	CUGAUGAGGCGGAAAGGCCGAA	AUCUUGA
276	UCCUGUG	CUGAUGAGGCGGAAAGGCCGAA	AGCCADU
301	CCAGGGA	CUGAUGAGGCGGAAAGGCCGAA	AUUCGAA
303	GACCAAG	CUGAUGAGGCGGAAAGGCCGAA	AGAUUUG
310	CCUUGGU	CUGAUGAGGCGGAAAGGCCGAA	ACCAGGG
323	UCAGGAG	CUGAUGAGGCGGAAAGGCCGAA	AGGGGCC
326	GGCCGGU	CUGAUGAGGCGGAAAGGCCGAA	AGGUGGA
335	UGUGGAU	CUGAUGAGGCGGAAAGGCCGAA	AGGCCGG
349	UCCCCAC	CUGAUGAGGCGGAAAGGCCGAA	AGUUCAU
352	GCUGUUC	CUGAUGAGGCGGAAAGGCCGAA	ADGAUCU
375	CUCAUAG	CUGAUGAGGCGGAAAGGCCGAA	AGCCAUU
376	CUCCGGA	CUGAUGAGGCGGAAAGGCCGAA	AGACCAU
378	AGCCUCA	CUGAUGAGGCGGAAAGGCCGAA	AGUAGCC
391	CUGGGCA	CUGAUGAGGCGGAAAGGCCGAA	AGGUCAG
409	AGCUAUG	CUGAUGAGGCGGAAAGGCCGAA	AUAUCUG
416	CUAUGGA	CUGAUGAGGCGGAAAGGCCGAA	ACTUGGG
417	GUUCUGG	CUGAUGAGGCGGAAAGGCCGAA	AGCUAUG
418	GGUUCUG	CUGAUGAGGCGGAAAGGCCGAA	AAGCUAU
433	CACACUG	CUGAUGAGGCGGAAAGGCCGAA	AUCCCCA
467	CGAACAG	CUGAUGAGGCGGAAAGGCCGAA	AGCCUGG
469	GCUGGCU	CUGAUGAGGCGGAAAGGCCGAA	AUGGCUU
473	CUGAUCU	CUGAUGAGGCGGAAAGGCCGAA	ACUCAAA
481	UGGUCUG	CUGAUGAGGCGGAAAGGCCGAA	AUUGGCU

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501	AACGUGA	CUGAUGAGGCOGAAAGGCOGAA	AGGGGUU
502	GAACGUG	CUGAUGAGGCOGAAAGGCOGAA	AAGGGGU
508	CUAUAGG	CUGAUGAGGCOGAAAGGCOGAA	ACGUGAA
509	UCUAUAG	CUGAUGAGGCOGAAAGGCOGAA	AACGUGA
512	UCCUCUA	CUGAUGAGGCOGAAAGGCOGAA	AGGAACG
514	GCUCUCU	CUGAUGAGGCOGAAAGGCOGAA	AUAGGAA
534	CAAGUCA	CUGAUGAGGCOGAAAGGCOGAA	AGUCCCC
556	GGAAGCA	CUGAUGAGGCOGAAAGGCOGAA	AGGCGCA
561	CACCUGG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGAG
562	UCACCUG	CUGAUGAGGCOGAAAGGCOGAA	AAGCAGA
585	GCUGGCU	CUGAUGAGGCOGAAAGGCOGAA	AUGGCUU
598	UCAGGAG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGCC
613	GUGAGAG	CUGAUGAGGCOGAAAGGCOGAA	ACAGGGG
616	GAUGUGA	CUGAUGAGGCOGAAAGGCOGAA	AGGACAG
617	GGCUGAG	CUGAUGAGGCOGAAAGGCOGAA	AAGGGAC
620	CAUGGCU	CUGAUGAGGCOGAAAGGCOGAA	AGGAAGG
623	GAGAUGG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGGA
628	UAUCAAA	CUGAUGAGGCOGAAAGGCOGAA	AUCGGAU
630	GUUAUCA	CUGAUGAGGCOGAAAGGCOGAA	AAAUCCG
631	GGUUAUC	CUGAUGAGGCOGAAAGGCOGAA	AAAADCG
638	GGAACAC	CUGAUGAGGCOGAAAGGCOGAA	AUGGCCA
661	AGAUUUU	CUGAUGAGGCOGAAAGGCOGAA	AGCUCCG
667	CUCCGCA	CUGAUGAGGCOGAAAGGCOGAA	AUCUUGA
687	GCUCCCA	CUGAUGAGGCOGAAAGGCOGAA	AGUCCCG
700	CCCCACC	CUGAUGAGGCOGAAAGGCOGAA	AGGCAGC
715	GCAAGAA	CUGAUGAGGCOGAAAGGCOGAA	AUCUCAU
717	CAGCAAG	CUGAUGAGGCOGAAAGGCOGAA	AGAUUCU
718	ACAGCAA	CUGAUGAGGCOGAAAGGCOGAA	AAGAUCU
721	CGCAADG	CUGAUGAGGCOGAAAGGCOGAA	AGGAGAA
751	ACACCCU	CUGAUGAGGCOGAAAGGCOGAA	AUGUCUU
759	CGUGAAA	CUGAUGAGGCOGAAAGGCOGAA	ACACCCU
761	CCCGUGA	CUGAUGAGGCOGAAAGGCOGAA	AUACACC
762	UCCCGUG	CUGAUGAGGCOGAAAGGCOGAA	AAUACAC
763	GUCCGGU	CUGAUGAGGCOGAAAGGCOGAA	AAAUACA
792	AGAAAAG	CUGAUGAGGCOGAAAGGCOGAA	AGCCUUG
795	UUGAGAA	CUGAUGAGGCOGAAAGGCOGAA	AGGAGCC
796	CUUGAGA	CUGAUGAGGCOGAAAGGCOGAA	AAGGAGC
797	GCUUGAG	CUGAUGAGGCOGAAAGGCOGAA	AAAGGAG
798	AGCUUGA	CUGAUGAGGCOGAAAGGCOGAA	AAAAGGA
829	GGAACAC	CUGAUGAGGCOGAAAGGCOGAA	AUGGCCA
834	AGUCCCG	CUGAUGAGGCOGAAAGGCOGAA	ACACAAU
835	GAGUCCG	CUGAUGAGGCOGAAAGGCOGAA	AACACAA
845	GCGUACG	CUGAUGAGGCOGAAAGGCOGAA	AGGAGUC
849	GUCCGCG	CUGAUGAGGCOGAAAGGCOGAA	ACGGAGG
872	CGAACAG	CUGAUGAGGCOGAAAGGCOGAA	AGCCUGG
883	GCAUGGA	CUGAUGAGGCOGAAAGGCOGAA	ACUCGAA
885	CUGCAUG	CUGAUGAGGCOGAAAGGCOGAA	AGACUCG
905	CGAUCAG	CUGAUGAGGCOGAAAGGCOGAA	AGGCCGC
906	GCGAUCA	CUGAUGAGGCOGAAAGGCOGAA	AAGGCCG

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919	GCUCACU	CUGAUGAGGCGGAAAGGCGCGAA	AGCUCGC
936	GUACUGG	CUGAUGAGGCGGAAAGGCGCGAA	ACUCCAU
937	AGUACUG	CUGAUGAGGCGGAAAGGCGCGAA	AACUCCA
942	UGGCAAG	CUGAUGAGGCGGAAAGGCGCGAA	ACUGGAA
953	UCAUGUG	CUGAUGAGGCGGAAAGGCGCGAA	AUGAGGC
962	CGGUGGC	CUGAUGAGGCGGAAAGGCGCGAA	ADCADCU
965	GUCUGGC	CUGAUGAGGCGGAAAGGCGCGAA	AGUACUG
973	UCUCUUC	CUGAUGAGGCGGAAAGGCGCGAA	ADCCGGU
986	ACUCUUG	CUGAUGAGGCGGAAAGGCGCGAA	AGGUCUC
996	GGUCUCA	CUGAUGAGGCGGAAAGGCGCGAA	AGGUCCU
1005	ACUCUUG	CUGAUGAGGCGGAAAGGCGCGAA	AGGUCUC
1006	UACUCUU	CUGAUGAGGCGGAAAGGCGCGAA	AAGGUCU
1015	UCUUCAU	CUGAUGAGGCGGAAAGGCGCGAA	AUACUCU
1028	UGGAAAG	CUGAUGAGGCGGAAAGGCGCGAA	ACUCUUC
1031	CCAUUGA	CUGAUGAGGCGGAAAGGCGCGAA	AGGACUC
1032	UCCAUUG	CUGAUGAGGCGGAAAGGCGCGAA	AAGGACU
1033	GUCCAUU	CUGAUGAGGCGGAAAGGCGCGAA	AAAGGAC
1058	CGGGUUG	CUGAUGAGGCGGAAAGGCGCGAA	AGGCGCG
1064	UUGGAUC	CUGAUGAGGCGGAAAGGCGCGAA	AGGUGUA
1072	GCACAGC	CUGAUGAGGCGGAAAGGCGCGAA	AUACGCC
1082	UUUCGGG	CUGAUGAGGCGGAAAGGCGCGAA	AGGCACA
1083	ACUUCGG	CUGAUGAGGCGGAAAGGCGCGAA	AAGGCUU
1092	AGAAGUU	CUGAUGAGGCGGAAAGGCGCGAA	AGUUUCG
1097	GGGACAG	CUGAUGAGGCGGAAAGGCGCGAA	AGUUGAG
1098	GGGGACA	CUGAUGAGGCGGAAAGGCGCGAA	AAGUUGA
1102	GCUUGGG	CUGAUGAGGCGGAAAGGCGCGAA	ACAGAAG
1125	GAAGGUG	CUGAUGAGGCGGAAAGGCGCGAA	AGGGCUG
1127	GUAAGGC	CUGAUGAGGCGGAAAGGCGCGAA	AUAUGGC
1131	UGGUGCU	CUGAUGAGGCGGAAAGGCGCGAA	AGGGADG
1132	AUGCUGG	CUGAUGAGGCGGAAAGGCGCGAA	AAGGUGU
1133	GAAGCUG	CUGAUGAGGCGGAAAGGCGCGAA	AGAUUGA
1137	GCGGCGU	CUGAUGAGGCGGAAAGGCGCGAA	AAGUAAA
1140	GCUGAGG	CUGAUGAGGCGGAAAGGCGCGAA	AUGCUGG
1153	CAAAGUU	CUGAUGAGGCGGAAAGGCGCGAA	AUGGUGC
1158	CUACUCA	CUGAUGAGGCGGAAAGGCGCGAA	AGUUGAU
1167	GGGGGAA	CUGAUGAGGCGGAAAGGCGCGAA	ACUCADC
1168	UGGGGGA	CUGAUGAGGCGGAAAGGCGCGAA	AACUCAU
1169	AUGGGGG	CUGAUGAGGCGGAAAGGCGCGAA	AAACUCA
1182	UGAUGGU	CUGAUGAGGCGGAAAGGCGCGAA	ACAGCAU
1183	CUGAUGG	CUGAUGAGGCGGAAAGGCGCGAA	AACAGCA
1184	UCAGGAG	CUGAUGAGGCGGAAAGGCGCGAA	AGGGGCC
1187	GGCUGAG	CUGAUGAGGCGGAAAGGCGCGAA	AAGGGAC
1188	CUGCCCU	CUGAUGAGGCGGAAAGGCGCGAA	AUGGUAA
1198	UCAGACU	CUGAUGAGGCGGAAAGGCGCGAA	AACUCCC
1209	GAAGGUG	CUGAUGAGGCGGAAAGGCGCGAA	AGGGCUG
1215	CGGUGCU	CUGAUGAGGCGGAAAGGCGCGAA	AGGCCAG
1229	GCUGAGG	CUGAUGAGGCGGAAAGGCGCGAA	AGGGACC
1237	GGGGCAG	CUGAUGAGGCGGAAAGGCGCGAA	AGCUGGG
1250	GAGCCUG	CUGAUGAGGCGGAAAGGCGCGAA	AGGCUGG

1268	GGGCGAG	CUGAUGAGGCGGAAAGGCGCGAA	AGCUGGG
1279	AGGAAGG	CUGAUGAGGCGGAAAGGCGCGAA	ACCAUGG
1281	CGCAGCU	CUGAUGAGGCGGAAAGGCGCGAA	AGGCCAC
1286	UGGGGGA	CUGAUGAGGCGGAAAGGCGCGAA	AACTCAU
1309	AGACUCG	CUGAUGAGGCGGAAAGGCGCGAA	ACAGGAG
1315	GGGUUAG	CUGAUGAGGCGGAAAGGCGCGAA	ACUGGGG
1318	CGGGGUU	CUGAUGAGGCGGAAAGGCGCGAA	AGAACUG
1331	GACUGGG	CUGAUGAGGCGGAAAGGCGCGAA	AGGACCC
1334	UCAGCUU	CUGAUGAGGCGGAAAGGCGCGAA	AGAAAAG
1389	GGCUUCC	CUGAUGAGGCGGAAAGGCGCGAA	ACAGCGU
1413	AGCAUCA	CUGAUGAGGCGGAAAGGCGCGAA	ACUGCAG
1414	CAGCAUC	CUGAUGAGGCGGAAAGGCGCGAA	AACTGCA
1437	GCCAAAG	CUGAUGAGGCGGAAAGGCGCGAA	AGGCCCC
1441	UGUUGCC	CUGAUGAGGCGGAAAGGCGCGAA	AGCAAGG
1467	GUCUGUG	CUGAUGAGGCGGAAAGGCGCGAA	ACACUCC
1468	GGUCUGU	CUGAUGAGGCGGAAAGGCGCGAA	AAACUUC
1482	GUCCACA	CUGAUGAGGCGGAAAGGCGCGAA	AUGCCAG
1486	AGUUCCC	CUGAUGAGGCGGAAAGGCGCGAA	ACCGAAG
1494	AAACUCU	CUGAUGAGGCGGAAAGGCGCGAA	AGUUGUC
1500	CUGCUGA	CUGAUGAGGCGGAAAGGCGCGAA	ACUCUGA
1501	GCUCUGG	CUGAUGAGGCGGAAAGGCGCGAA	AACTCUG
1502	AGCUGCU	CUGAUGAGGCGGAAAGGCGCGAA	AAACUCU
1525	ACACAGG	CUGAUGAGGCGGAAAGGCGCGAA	AUGCACC
1566	UUCAGGG	CUGAUGAGGCGGAAAGGCGCGAA	ACTGCCU
1577	CGAGUUA	CUGAUGAGGCGGAAAGGCGCGAA	AGCUUCA
1579	GGCGAGU	CUGAUGAGGCGGAAAGGCGCGAA	AUAGCUU
1583	ACCAAGG	CUGAUGAGGCGGAAAGGCGCGAA	AGUUAUA
1588	CCUCUCU	CUGAUGAGGCGGAAAGGCGCGAA	AGGAGAG
1622	GGGCGAG	CUGAUGAGGCGGAAAGGCGCGAA	AGCUGGG
1628	CCUACCG	CUGAUGAGGCGGAAAGGCGCGAA	AGCAGGA
1648	CAUUGGG	CUGAUGAGGCGGAAAGGCGCGAA	AGCCCGG
1660	CUGGGCA	CUGAUGAGGCGGAAAGGCGCGAA	AGGUCAG
1663	CACUUGG	CUGAUGAGGCGGAAAGGCGCGAA	AGCAGAG
1664	UCACCUG	CUGAUGAGGCGGAAAGGCGCGAA	AAGCAGA
1665	ACCUCCG	CUGAUGAGGCGGAAAGGCGCGAA	AAGCGAG
1680	GGAGGAG	CUGAUGAGGCGGAAAGGCGCGAA	AGUCUUC
1681	UGGAGGA	CUGAUGAGGCGGAAAGGCGCGAA	AAGUCUU
1683	AADGGAG	CUGAUGAGGCGGAAAGGCGCGAA	AGAAGUC
1686	CGCAADG	CUGAUGAGGCGGAAAGGCGCGAA	AGGAGAA
1690	UGUCCGC	CUGAUGAGGCGGAAAGGCGCGAA	AUGGAGG
1704	AGCAGAG	CUGAUGAGGCGGAAAGGCGCGAA	AGUCCAU
1705	GAGCAGA	CUGAUGAGGCGGAAAGGCGCGAA	AAGUCCA
1707	AAGAGCA	CUGAUGAGGCGGAAAGGCGCGAA	AGAAGUC
1721	CUGAUCU	CUGAUGAGGCGGAAAGGCGCGAA	ACUCAAA
1726	AGGAGCU	CUGAUGAGGCGGAAAGGCGCGAA	AUCUGAC
1731	ACCUUAG	CUGAUGAGGCGGAAAGGCGCGAA	AGCUGAU
1734	AGCACCU	CUGAUGAGGCGGAAAGGCGCGAA	AGGAGCU
1754	CUCUUGG	CUGAUGAGGCGGAAAGGCGCGAA	AGCACUG

Table 20
Human rel A HH Ribozyme Sequences
nt. Position HH Ribozyme Sequences

19	UACAGAC	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
22	CACUACA	CUGAUGAGGCCGAAAGGCCGAA	ACGAGCC
26	CGUGCAC	CUGAUGAGGCCGAAAGGCCGAA	ACAGACG
93	GAGGGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAGUUC
94	UGAGGGG	CUGAUGAGGCCGAAAGGCCGAA	AACAGUU
100	GGAAGAU	CUGAUGAGGCCGAAAGGCCGAA	AGGGGGA
103	CCGGGAA	CUGAUGAGGCCGAAAGGCCGAA	ADGAGGS
105	UGCGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAG
106	CUGCGGG	CUGAUGAGGCCGAAAGGCCGAA	AAGADGA
129	GGGGCCA	CUGAUGAGGCCGAAAGGCCGAA	AGGCGUG
138	CUCCACA	CUGAUGAGGCCGAAAGGCCGAA	AGGGGOC
148	GCUCAAU	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCA
151	GCUGCUC	CUGAUGAGGCCGAAAGGCCGAA	ADGAUCU
180	GUAGCGG	CUGAUGAGGCCGAAAGGCCGAA	AGCGCAU
181	UGUAGCG	CUGAUGAGGCCGAAAGGCCGAA	AAGCGCA
186	GCACUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCGGAA
204	GCCCGCG	CUGAUGAGGCCGAAAGGCCGAA	AGCGGCC
217	CGCCUGG	CUGAUGAGGCCGAAAGGCCGAA	ADGCGGC
239	UUGGUGG	CUGAUGAGGCCGAAAGGCCGAA	ADGUGUG
262	UGAUCUU	CUGAUGAGGCCGAAAGGCCGAA	ADGGUGG
268	AGCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGA
276	UCCUGUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
301	CCAGGGA	CUGAUGAGGCCGAAAGGCCGAA	ADGCGCA
303	GACCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGGG
310	CCUUGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAGGG
323	CGGUGAG	CUGAUGAGGCCGAAAGGCCGAA	AGGGUCC
326	GGCCGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGAGGG
335	UGGGGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCCGG
349	UUCCUAC	CUGAUGAGGCCGAAAGGCCGAA	AGCUUGU
352	CCUUUCC	CUGAUGAGGCCGAAAGGCCGAA	ACAAGCU
375	CUCAUAG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAUU
376	CCUCAUA	CUGAUGAGGCCGAAAGGCCGAA	AAGCCAU
378	AGCCUCA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGCC
391	CCGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCAG
409	AACUGUG	CUGAUGAGGCCGAAAGGCCGAA	ADGCMGC
416	UUCUGGA	CUGAUGAGGCCGAAAGGCCGAA	ACUGUGG
417	GUUCUGG	CUGAUGAGGCCGAAAGGCCGAA	AACUGUG
418	GGUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAACUGU
433	CACACUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCCA
467	UGACUGA	CUGAUGAGGCCGAAAGGCCGAA	AGCCUGC
469	GCUGACT	CUGAUGAGGCCGAAAGGCCGAA	ADAGCCU
473	AUGCGCU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAUA
481	UGGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AUGCGCU
501	AACUUGG	CUGAUGAGGCCGAAAGGCCGAA	AGGGGUU

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502	GAACUUG	CUGAUGAGGCOGAAAGGCOGAA	AAGGGGU
508	CUAATAGG	CUGAUGAGGCOGAAAGGCOGAA	ACUUGGA
509	UCUAUAG	CUGAUGAGGCOGAAAGGCOGAA	AACUUGG
512	UCUUCUA	CUGAUGAGGCOGAAAGGCOGAA	AGGAACU
514	GCUCUUC	CUGAUGAGGCOGAAAGGCOGAA	AUAGGAA
534	CAGGUUG	CUGAUGAGGCOGAAAGGCOGAA	AGUCCUC
556	GGAAACA	CUGAUGAGGCOGAAAGGCOGAA	AGCCGCA
561	CACCUUG	CUGAUGAGGCOGAAAGGCOGAA	AGCAGAG
562	UCACCUG	CUGAUGAGGCOGAAAGGCOGAA	AAGCAGA
585	CCUGCCU	CUGAUGAGGCOGAAAGGCOGAA	AUGGGUC
598	GCAGGCG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGCC
613	GAGGAAG	CUGAUGAGGCOGAAAGGCOGAA	ACAGGCG
616	GADGAGG	CUGAUGAGGCOGAAAGGCOGAA	AGGACAG
617	GGADGAG	CUGAUGAGGCOGAAAGGCOGAA	AAGGACA
620	AUGGGAU	CUGAUGAGGCOGAAAGGCOGAA	AGGAAGG
623	AAGAUGG	CUGAUGAGGCOGAAAGGCOGAA	AUGAGGA
628	UGUCAAA	CUGAUGAGGCOGAAAGGCOGAA	AUGGGAU
630	AUUGUCA	CUGAUGAGGCOGAAAGGCOGAA	AGADGGG
631	GADUGUC	CUGAUGAGGCOGAAAGGCOGAA	AAGAUGG
638	GGGGCAC	CUGAUGAGGCOGAAAGGCOGAA	AUUGUCA
661	AGADCUU	CUGAUGAGGCOGAAAGGCOGAA	AGCUUGG
667	CUUGGCA	CUGAUGAGGCOGAAAGGCOGAA	AUCUUGA
687	GCUGCCA	CUGAUGAGGCOGAAAGGCOGAA	AGUUUUG
700	CCCCACC	CUGAUGAGGCOGAAAGGCOGAA	AGGCAGC
715	GUAGGAA	CUGAUGAGGCOGAAAGGCOGAA	AUCUCAU
717	CAGUAGG	CUGAUGAGGCOGAAAGGCOGAA	AGADUUC
718	ACAGUAG	CUGAUGAGGCOGAAAGGCOGAA	AAGADCU
721	CACACAG	CUGAUGAGGCOGAAAGGCOGAA	AGGAAGA
751	ACACCUU	CUGAUGAGGCOGAAAGGCOGAA	AUGUCCU
759	CGUGAAA	CUGAUGAGGCOGAAAGGCOGAA	ACACCUU
761	CCCGUGA	CUGAUGAGGCOGAAAGGCOGAA	AUACACC
762	UCCCGUG	CUGAUGAGGCOGAAAGGCOGAA	AAUACAC
763	GUCCCGU	CUGAUGAGGCOGAAAGGCOGAA	AAAUACA
792	CGAAAAG	CUGAUGAGGCOGAAAGGCOGAA	AGCCUUG
795	UUGCGAA	CUGAUGAGGCOGAAAGGCOGAA	AGGAGCC
796	CUUGCGA	CUGAUGAGGCOGAAAGGCOGAA	AAGGAGC
797	GCUUGCG	CUGAUGAGGCOGAAAGGCOGAA	AAAGGAG
798	AGCUUGC	CUGAUGAGGCOGAAAGGCOGAA	AAAAGGA
829	GGAAACAC	CUGAUGAGGCOGAAAGGCOGAA	AUGGCCA
834	GGUCCGG	CUGAUGAGGCOGAAAGGCOGAA	ACACAAU
835	GGGUCCG	CUGAUGAGGCOGAAAGGCOGAA	AACACAA
845	GCGUAGG	CUGAUGAGGCOGAAAGGCOGAA	AGGGGUC
849	GUCUGCG	CUGAUGAGGCOGAAAGGCOGAA	AGGGAGG
872	CGCACAG	CUGAUGAGGCOGAAAGGCOGAA	AGCCUUC
883	GCAUGGA	CUGAUGAGGCOGAAAGGCOGAA	ACACGCA
885	CUGCAUG	CUGAUGAGGCOGAAAGGCOGAA	AGACACG
905	CGGUCCG	CUGAUGAGGCOGAAAGGCOGAA	AGGCGGC
906	CCGGUUG	CUGAUGAGGCOGAAAGGCOGAA	AAGGCGG
919	GCUCAAU	CUGAUGAGGCOGAAAGGCOGAA	AGCUCCC

936	GUACUGG	CUGAUGAGGCGGAAAGGCCGAA	AUUCCAU
937	GGUACUG	CUGAUGAGGCGGAAAGGCCGAA	AAUCCA
942	UGGCAGG	CUGAUGAGGCGGAAAGGCCGAA	ACUGGAA
953	UCGUCUG	CUGAUGAGGCGGAAAGGCCGAA	AUCUGGC
962	CGGUGAC	CUGAUGAGGCGGAAAGGCCGAA	AUGGUCU
965	AUCCGGU	CUGAUGAGGCGGAAAGGCCGAA	ACGAUUG
973	UCUCCUC	CUGAUGAGGCGGAAAGGCCGAA	AUCCGGU
986	GUCCUUU	CUGAUGAGGCGGAAAGGCCGAA	ACGUUUC
996	GGUCUCA	CUGAUGAGGCGGAAAGGCCGAA	AUGGUCU
1005	GCUCUUG	CUGAUGAGGCGGAAAGGCCGAA	AGGUCUC
1006	UGCUCUU	CUGAUGAGGCGGAAAGGCCGAA	AAGGUCU
1015	UCUUCAU	CUGAUGAGGCGGAAAGGCCGAA	AUGGUCU
1028	CUGAAAG	CUGAUGAGGCGGAAAGGCCGAA	ACUCUUC
1031	CCGCUGA	CUGAUGAGGCGGAAAGGCCGAA	AGGACUC
1032	UCGCGUG	CUGAUGAGGCGGAAAGGCCGAA	AAGGACU
1033	GUCCGCU	CUGAUGAGGCGGAAAGGCCGAA	AAAGGAC
1058	CGAGGUG	CUGAUGAGGCGGAAAGGCCGAA	AGGCCGG
1064	AUGGUGC	CUGAUGAGGCGGAAAGGCCGAA	AGGUGGA
1072	GCACAGC	CUGAUGAGGCGGAAAGGCCGAA	AUGGUCU
1082	CUGGGGG	CUGAUGAGGCGGAAAGGCCGAA	AGGCACA
1083	GCUGGGG	CUGAUGAGGCGGAAAGGCCGAA	AAGGCAC
1092	AGAAGCU	CUGAUGAGGCGGAAAGGCCGAA	AGCUGCG
1097	GGGACAG	CUGAUGAGGCGGAAAGGCCGAA	AGCUGAG
1098	GGGACA	CUGAUGAGGCGGAAAGGCCGAA	AAGCUGA
1102	GCUGGGG	CUGAUGAGGCGGAAAGGCCGAA	ACAGAAG
1125	AAAGGGA	CUGAUGAGGCGGAAAGGCCGAA	AGGGCUG
1127	GUAAAGG	CUGAUGAGGCGGAAAGGCCGAA	AUAGGGC
1131	UGACGUA	CUGAUGAGGCGGAAAGGCCGAA	AGGGADA
1132	AUGACGU	CUGAUGAGGCGGAAAGGCCGAA	AAGGGAU
1133	GAUGACG	CUGAUGAGGCGGAAAGGCCGAA	AAAGGGA
1137	CAGGGAU	CUGAUGAGGCGGAAAGGCCGAA	ACGUAAA
1140	GCUCAGG	CUGAUGAGGCGGAAAGGCCGAA	AUGACGU
1153	CAUAGUU	CUGAUGAGGCGGAAAGGCCGAA	AUGGUGC
1158	CUCAUCA	CUGAUGAGGCGGAAAGGCCGAA	AGUUGAU
1167	GGUGGGA	CUGAUGAGGCGGAAAGGCCGAA	ACUCAUC
1168	UGGUGGG	CUGAUGAGGCGGAAAGGCCGAA	AACUCAU
1169	AUGGUGG	CUGAUGAGGCGGAAAGGCCGAA	AAACUCA
1182	AGAAGGA	CUGAUGAGGCGGAAAGGCCGAA	ACACCAU
1183	CAGAAGG	CUGAUGAGGCGGAAAGGCCGAA	AACACCA
1184	CCAGAAG	CUGAUGAGGCGGAAAGGCCGAA	AAACACC
1187	UGCCAG	CUGAUGAGGCGGAAAGGCCGAA	AGGAAAC
1188	CUGCCCA	CUGAUGAGGCGGAAAGGCCGAA	AAGGAAA
1198	CCUGGCU	CUGAUGAGGCGGAAAGGCCGAA	AUCUGCC
1209	CAAGGCC	CUGAUGAGGCGGAAAGGCCGAA	AGGCCUG
1215	CGGGGCC	CUGAUGAGGCGGAAAGGCCGAA	AGGCCGA
1229	ACUUGGG	CUGAUGAGGCGGAAAGGCCGAA	AGGGGCC
1237	GGGGCAG	CUGAUGAGGCGGAAAGGCCGAA	ACUUGGG
1250	GGGGCUG	CUGAUGAGGCGGAAAGGCCGAA	AGCCUGG
1268	AUGGCCG	CUGAUGAGGCGGAAAGGCCGAA	AGCAGGG

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1279	GAGCUGA	CUGAUGAGGCGAAAGGCGGAA	ACCAUGG
1281	CAGAGCU	CUGAUGAGGCGAAAGGCGGAA	AUACCAU
1286	UGGGCCA	CUGAUGAGGCGAAAGGCGGAA	AGCUGAU
1309	GGACUGG	CUGAUGAGGCGAAAGGCGGAA	ACAGGGG
1315	GGGCUAG	CUGAUGAGGCGAAAGGCGGAA	ACUGGGA
1318	CUGGGGC	CUGAUGAGGCGAAAGGCGGAA	AGGACUG
1331	GCCUGAG	CUGAUGAGGCGAAAGGCGGAA	AGGGCCU
1334	ACAGCCU	CUGAUGAGGCGAAAGGCGGAA	AGGAGGG
1389	GGCCUCU	CUGAUGAGGCGAAAGGCGGAA	ACAGCGU
1413	AUCAUCA	CUGAUGAGGCGAAAGGCGGAA	ACUCGAG
1414	CAUCAUC	CUGAUGAGGCGAAAGGCGGAA	AACUGCA
1437	GCCAAGC	CUGAUGAGGCGAAAGGCGGAA	AGGCCCC
1441	UGUUGCC	CUGAUGAGGCGAAAGGCGGAA	AGCRAAG
1467	GUCUGUG	CUGAUGAGGCGAAAGGCGGAA	ACACAGC
1468	GGCCUGU	CUGAUGAGGCGAAAGGCGGAA	AACACAG
1482	GUUGAUG	CUGAUGAGGCGAAAGGCGGAA	AUGCCAG
1486	AGUUGUC	CUGAUGAGGCGAAAGGCGGAA	ACCGAUG
1494	AAACUUG	CUGAUGAGGCGAAAGGCGGAA	AGUUGUC
1500	CUGCUGA	CUGAUGAGGCGAAAGGCGGAA	ACUUGGA
1501	GCCGCGG	CUGAUGAGGCGAAAGGCGGAA	AACUUGG
1502	AGCUGCU	CUGAUGAGGCGAAAGGCGGAA	AAACUUG
1525	CCACAGG	CUGAUGAGGCGAAAGGCGGAA	AUGCCCU
1566	CUCAGGG	CUGAUGAGGCGAAAGGCGGAA	ACUCCAU
1577	CGAGUUA	CUGAUGAGGCGAAAGGCGGAA	AGCCUCA
1579	GGGAGU	CUGAUGAGGCGAAAGGCGGAA	AUAGCCU
1583	ACUAGGC	CUGAUGAGGCGAAAGGCGGAA	AGUUAUA
1588	CUGUCAC	CUGAUGAGGCGAAAGGCGGAA	AGGCGAG
1622	GGAGCAG	CUGAUGAGGCGAAAGGCGGAA	AGCUGGG
1628	CCAGGUG	CUGAUGAGGCGAAAGGCGGAA	AGCAGGA
1648	CAUUGGG	CUGAUGAGGCGAAAGGCGGAA	AGGCCCG
1660	CUGAAG	CUGAUGAGGCGAAAGGCGGAA	AGGCCAU
1663	CUCCUGA	CUGAUGAGGCGAAAGGCGGAA	AGGAGGC
1664	UCUCCUG	CUGAUGAGGCGAAAGGCGGAA	AAGGAGG
1665	AUCUCCU	CUGAUGAGGCGAAAGGCGGAA	AAAGGAG
1680	GGAGGAG	CUGAUGAGGCGAAAGGCGGAA	AGUCUUC
1681	UGGAGGA	CUGAUGAGGCGAAAGGCGGAA	AAGDCUU
1683	AADGGAG	CUGAUGAGGCGAAAGGCGGAA	AGAAGUC
1686	CGCAUUG	CUGAUGAGGCGAAAGGCGGAA	AGGAGAA
1690	UGUCCGC	CUGAUGAGGCGAAAGGCGGAA	AUGGAGG
1704	GGCUGAG	CUGAUGAGGCGAAAGGCGGAA	AGUCCAU
1705	GGCUGA	CUGAUGAGGCGAAAGGCGGAA	AAGUCCA
1707	CAGGGCU	CUGAUGAGGCGAAAGGCGGAA	AGAAGUC
1721	CUGAUCU	CUGAUGAGGCGAAAGGCGGAA	ACUCAGC
1726	AGGAGCU	CUGAUGAGGCGAAAGGCGGAA	AUCUGAC
1731	CCCUUAG	CUGAUGAGGCGAAAGGCGGAA	AGCUGAU
1734	ACCCUCC	CUGAUGAGGCGAAAGGCGGAA	AGGAGCU
1754	CUCUGGG	CUGAUGAGGCGAAAGGCGGAA	AGGCGAG

Human re/A Hairpin Ribozyme/Target Sequences

[illegible]

Position	Halpripin Ribozyme sequence	Substrate
90	UGAGGGGG AGAA GUUC ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	GAACU GUU CCCCCUA
156	GCUCGUUG AGAA GCUC ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	GAGCA GCC CAAAGCAGC
362	GCCAUCCG AGAA GUCC ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	GAACU GCC GGAUGGCG
413	GUUCUGGA AGAA GUUG ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	CCACA GUU UCCAGAAC
606	GAAGGACA AGAA GCAG ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	CGGCC GUC UGUCCUUC
652	UUGAGUCU AGAA GUGU ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	ACACU GCC GAGCUCUA
695	CCCACCCA AGAA GCUG ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	CACGU GCC UCCAGUGG
853	AGGCUUGG AGAA GCGU ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	ACCCA GAC CCGAGCCU
900	GGUCGGAA AGAA GCGG ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	CGGGG GCC UUDCGACC
955	UGACGAUC AGAA GUUU ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	AUACA GAC GAUCGUCA
11037	GUCGUGUG AGAA GCUU ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	CAGGG GAC CCAACGAC
11045	GGCCGGGG AGAA GUUG ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	CCACC GAC CCGCGGCC
14410	CAUCAUCA AGAA CCAG ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	CUCCA GUU UGAUGAUG
14453	ACAGCUGG AGAA GUCC ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	GCACA GAC CCAAGCUGU
1471	GAUGCCAG AGAA GUZA ACCAGAGAAACACACAGUUGUGGUA CAUUAACCUUGUA	UCACA GAC CUGGCAUC

Table 22
 Mouse *rel*/A Hairpin Ribozyme/Target Sequences
 nt. Position Hairpin Ribozyme sequence

		Substrate
137	GUUGCUC AGAA GUUC ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	GAACA GCC GAAGCAAC
273	GAGAUUG AGAA GUUC ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	GAACA GUU CGAAUCUC
343	GCCAUCC AGAA GUUC ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	GAACU GCC GGGUUGGC
366	GCGCAGAG AGAA GCUU ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	AGCCU GAC CUCUGCCC
633	UUGAGCUC AGAA GUGU ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	ACACU GCC GAACUCAA
676	CCCAACCA AGAA GCUU ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	GAACU GCC UCGUUGGG
834	AGGCUGG AGAA GCUU ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	ACGCC GAC CCCAGCCU
881	GAUCAGAA AGAA GCGG ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	CGCGG GCC UUCUGAUC
1100	AGGUGAG AGAA GCGG ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	CCGCA GCC CUAACACU
1205	GCGCAGAG AGAA GUUC ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	GCACC GUC CUCUGCCC
1361	GCGCUUC AGAA GCGU ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	ACCCU GUC GGAAGCCG
1385	CAGCAUCA AGAA GCGG ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	CUGCA GUU UGAUGCUG
1431	ACTUCCUG AGAA GUUC ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	GCACA GAC GCGGAGAU
1449	GAUGCCAG AGAA GUGA ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	UCACA GAC CUGGCAUC
1802	AAGUCCG AGAA GCUU ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	CAGCU GCC CCCGACUU
2009	UGGCUCA AGAA GUUC ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	GGACA GAC UGGAGCCA
2124	UGGUGUG AGAA GCGC ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	GUCCU GCC CGACACCA
2233	AUUCUGAA AGAA GCGA ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	UGGCC GCC UUCGAAU
2354	UCAGUAAA AGAA GUCU ACCAGAGAAAACACACAGUUGUGGUACAUUACCUUGUA	AGACA GCC UUUACUGA

Table 23: Human TNF- α HH Ribozyme Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
28	GGCAGGU U CUCUUC	321	GUCAGAU C AUCUUCU
29	GCAGGUU C UCUUCCU	324	AGAUCAU C UUCUGA
31	AGGUUCU C UUCUCU	326	AUCAUU U CUCGAAC
33	GUUCUU U CCUCUA	327	UCAUCU C UCGAAC
34	UUCUUU C CUCUCAC	329	AUCUUCU C GACCCC
37	UCUUCU C UCACUA	352	AGCCUGU A GCCCAG
39	UUCUCU C ACUAU	361	CCCAUGU U GUAGCA
44	CUCACAU A CUGACC	364	AUGUUGU A GCAAAC
58	CACGGU C CACCCU	374	AAACCCU C AAGCUA
65	CCACCU C UCUCCC	391	GGCAGU C CAGUGG
67	ACCCCU C UCCCUU	421	AUGCCU C CUGGCA
69	CCUCUU C CCGGGA	449	GAGAGAU A ACCCAU
106	GCAUGAU C CGGACG	468	GUGCAU C AGAGGC
136	AGGGCU C CCAAGA	480	GGCCUGU A CCUCAC
165	CAGGGU C CAGGGG	484	UGUAUU C AUCUACU
177	CGGUCU U GUUCUC	487	ACCUAU C UACUCC
180	UGCUUGU U CCUCAG	489	CUCAUU A CUCACG
181	GCUGUU C CUCAGC	492	AUCUACU C CCAGUC
184	UGUUCU C AGCUCU	499	CCAGGU C CUCUCA
190	UCAGCU C UUCUCU	502	AGGUUU C UUCAAG
192	AGCCCU U CUCUUC	504	GUUUUU U CAAGGC
193	GCCUUU C UCUUCC	505	UCCUUU C AAGGGC
195	CUCUUU C CUCUUG	525	UGCCCU C CACCAU
198	UUCUUU U CUCGAC	538	AUGUGU C CUCACC
199	UCUUCU C CUGACG	541	UGCUUU C ACCACA
205	UCCGAU C GUGCAG	553	ACACCAU C AGCGCA
226	CCAGCU C UUCUGC	562	GGGCAU C GCGUCU
228	ACGCUU U CUGCUG	568	UGGCGU C UCCUAC
229	CGCUCU C UGCUGC	570	GCGUCU C CUAACG
243	CUGCAU U UGGAGU	573	GUCUUU A CCAGAC
244	UGCACU U GGAGUA	586	CCAAGGU C AACCUU
253	GAGUAU C GGGCCC	592	UCAACU C CUCUCU
273	GAAGAU C CCCCAG	595	ACCUUU C UCUGCA
286	GGGACU C UCUCUA	597	CUCUUU C UGCACU
288	GACCUU C UCUAAC	604	CUGCCAU C AAGAGC
290	CCUCUU C UAACAG	657	CCUGGU A UGAGCC
292	UCUCUU A AUCAGC	667	AGCCAU C UAUUGG
295	CUCUAU C AGCCUC	669	CCCAU A UCUGGA
302	CAGCCU C UGGCCA		

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671	CAUCUAT C UGGGAGG	960	UGGGAUU C AGGAUUG
682	GAGGGGU C UUCACAG	1001	AACCACU A AGAAUUC
684	GGGGUUC U CCAGCUG	1007	UAAGAAU U CAAACUG
685	GGGUUCU C CAGCUGG	1008	AAGAAUU C AAACUGG
709	ACCGACU C AGGCGUG	1021	GGGGCCU C CAGAACU
721	CUGAGAU C AAUCGGC	1029	CAGAACU C ACUGGGG
725	GAUCAAU C GGCCCGA	1040	GGGGCCU A CAGCUUU
735	CCCGACU A UCCCGAC	1046	UACAGCU U UGADCCC
737	CGACTAU C UCGACUU	1047	ACAGCUU U GAUCCCU
739	ACUATCU C GACUUUG	1051	CUUGAUU C CUGACA
744	CUCGACU U UGCGAG	1060	CUGACAU C UGGAUUC
745	UUGACUU U GCGAGU	1067	CUGGAUU C UGGAGAC
753	GCGAGU C UGGGCGG	1085	GGAGCCU U UGGUUCU
763	GGCAGGU C UACUUUG	1086	GAGCCUU U GGUUCUG
765	CAGGUUC A CUUUGGG	1090	CUUUGGU U CUGGCCA
768	GUCUACU U UGGGAUC	1091	UUUGGUU C UGGCCAG
769	UCUACUU U GGGAUCA	1113	CAGGACU U GAGAAGA
775	UUGGGAU C AUUGCCC	1124	AAGACCU C ACCUAGA
778	GGADCAU U GCGCUGU	1129	CUACCCU A GAAAUUG
801	CGAACAU C CAACCUU	1135	UAGAAAU U GACACAA
808	CCAACCU U CCCAARC	1151	UGGACCU U AGGCCUU
809	CAACCUU C CCAAACG	1152	GGACCUU A GGCCUUC
820	AAAGCCU C CCGGCC	1158	UAGGCCU U CCGCCUU
833	CCCAAU C CCUUUAU	1159	AGGCCUU C CUCUCUC
837	AADCCCU U UAUUACC	1162	CCUUCUU C UCCCCAG
838	AUCCCUU U AUUACCC	1164	UCCCUUU C UCCAGAU
839	UCCCUUU A UUAACCC	1166	CCUUCUU C CAGAUGU
841	CCUUUAU U ACCCCCU	1174	CAGAUGU U UCCAGAC
842	CUUUUAU A CCCCCUC	1175	AGAUGUU U CCAGACU
849	ACCCCCU C CUUCAGA	1176	GAUGUUU C CAGACUU
852	CCUCCCU U CAGACAC	1183	CCAGACU U CCUUGAG
853	CCUCCCU C AGACACC	1184	CAGACUU C CUUGAGA
863	ACACCCU C AACCCCU	1187	ACUUCUU U GAGACAC
869	UCRACCU C UUCUGGC	1208	CAGCCCU C CCAUGG
871	AAACCUU U CUGGCUC	1224	GCCAGCU C CCUCUAU
872	ACCUCUU C UGGCUCA	1228	GCUCUUU C UAUUUUU
878	UCUGGCU C AAAAAGA	1230	UCCCUUU A UUUUUGU
890	AGAGAAU U GGGGGCU	1232	CCUCUAU U UAGGUUU
898	GGGGGCU U AGGGUUG	1233	CUUCUAU U AUGGUUG
899	GGGGCUU A GGGUUGG	1234	UCUAUUU A UGUUUGC
904	UUGGGU C GGAACCC	1238	UUUAGUU U UGCACUU
917	CCAAGCU U AGAACUU	1239	UUUAGUU U GCACUUG
918	CAAGCUU A GAACUUU	1245	UUGCACU U GUGAUUA
924	UAGAACU U UAAGCAA	1251	UUGUGAU U AUUUUUU
925	AGAACUU U AAGCAAC	1252	UGUGAUU A UUUUUUA
926	GAACUUU A AGCAACA	1254	UGAUUAU U UAUUAUU
945	CACCACU U CGAAACC	1255	GAUUUUU U AUUAUUU
946	ACCACUU C GAAACCU	1256	AUUUUUU A UUAUUUA
959	CUGGGAU U CAGGAU	1258	UAUUUAU U AUUUUUU

1259	AUUUAUU A UUUUAUU	1440	UGUUUUU U AAAAUUU
1261	UUUUUUU U UUUUUUU	1441	GUUUUUU A AAAAUUU
1262	UUUUUUU U AUUUUUU	1446	UUAAAAU A UUAUCUG
1263	AUUUAUU A UUUUAUU	1448	AAAAUUU U AUUGAUU
1265	UUUUUUU U UUUUUUU	1449	AAAAUUU A UUGUAUU
1266	AUUUAUU U AUUUUUU	1451	AUUUAUU C UGAUUAA
1267	UUUUUUU A UUUUUUU	1456	AUUGAUU U AAGUUGU
1269	UUUUUUU U AUUUUUU	1457	UUGUAUU A AGUUGUC
1270	AUUUAUU A UUUUUUU	1461	AUUUAUU U GUUUAAA
1272	UUUUUUU U UUUUUUU	1464	AAGUUGU C UAAACAA
1273	UUUUUUU U AUUUUUU	1466	GUUGGUU A AACAADG
1274	AUUUAUU A UUUUUUU	1479	UGUGAUU U UGUGGAC
1276	UUUUUUU U UUUUUUU	1480	GUUGAUU U GUUGACC
1277	AUUUAUU U AUUUUUU	1494	CUACUGU C ACUGAUU
1278	UUUUUUU A UUUUUUU	1498	UGUCACU C AUUGGUG
1280	UUUUUUU U UACAGAU	1501	CACUCAU U GUUGAGG
1281	AUUUAUU U ACAGADG	1512	GAGGUUU C UGUUUUU
1282	UUUUUUU A CAGADGA	1517	CUUGGUU C UUGAGGG
1294	UGAAGUU A UUUUUUU	1528	AGGGAGU U GUUGGUG
1296	AUUGAUU U UAUUGG	1533	GUUGGUU C UGUUADC
1297	AUGUAUU U AUUGGG	1537	UGUGGUU A AUUGGUC
1298	UGUAUUU A UUGGGA	1540	CUGUUAD C GUUUUAC
1300	UUUUUUU U UGGGGA	1546	UGGGGUU A CUUUUCA
1301	AUUUAUU U GGGGAC	1549	GUUUUACU A UUGAGUG
1315	UGGGGUU A UUGGGG	1551	CUUUUACU U CAGUGGC
1317	GGGUUUU C UUGGGG	1552	UACUUUU C AGUGGUG
1334	CUUUUUU A GUUGGUG	1566	GAGAAAU A AAGGUUG
1345	GUUGGUU U GUUGGUG	1572	UAAAGGU U GUUUAGG
1350	CUUGGUU C AGACADG	1576	GUUGGUU U AGGAAAG
1359	GACADGU U UUGGUG	1577	GUUGGUU A GGAAGA
1360	ACADGUU U UUGGUG		
1361	CAUGUUU U UUGGUG		
1362	AUGUUUU C UUGGUG		
1386	GAACAAU A GUUGGUU		
1393	AGGUGGU U UUGGUG		
1394	GUUGGUU C UUGGUG		
1401	CUUUUUU A GUUGGUU		
1414	CUUGGUU C UUGGUG		
1422	UGUGGUU U CUUUUGA		
1423	GUUGGUU C UUUUGAU		
1425	GUUUUUU U UUGAUUA		
1426	CUUUUUU U UGAUUUU		
1427	CUUUUUU U GAUUUUG		
1431	UUUUUUU U AUUUUUU		
1432	UUUUUUU A UUUUUUU		
1436	AUUUAUU U UUUUUUU		
1437	UUUUUUU U UUUUUUU		
1438	UUUUUUU U UUUUUUU		

Table 24: Human TNF- α Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
28	GGAAGAG CUGAUGAGGCGGAAAGGCGGAA ACCUGCC
29	AGGAAGA CUGAUGAGGCGGAAAGGCGGAA AACCUCC
31	AGAGGA CUGAUGAGGCGGAAAGGCGGAA AGAACCU
33	UGAGAGG CUGAUGAGGCGGAAAGGCGGAA AGAGAAC
34	GUGAGAG CUGAUGAGGCGGAAAGGCGGAA AAGAGAA
37	UADUGA CUGAUGAGGCGGAAAGGCGGAA AGGAAGA
39	AGUADGU CUGAUGAGGCGGAAAGGCGGAA AGAGGAA
44	GGGUCAG CUGAUGAGGCGGAAAGGCGGAA AUGUGAG
58	GAGGGUG CUGAUGAGGCGGAAAGGCGGAA AGCGGUG
65	GGGGAGA CUGAUGAGGCGGAAAGGCGGAA AGGGGGG
67	CAGGGGA CUGAUGAGGCGGAAAGGCGGAA AGAGGGU
69	UCCAGGG CUGAUGAGGCGGAAAGGCGGAA AGAGAGG
106	CGUCCCG CUGAUGAGGCGGAAAGGCGGAA AUCADGC
136	UCUUGGG CUGAUGAGGCGGAAAGGCGGAA AGCGCCU
165	CCGCCUG CUGAUGAGGCGGAAAGGCGGAA AGCCCCU
177	GAGGAAC CUGAUGAGGCGGAAAGGCGGAA AGCACCG
180	GCUGAGG CUGAUGAGGCGGAAAGGCGGAA ACAAGCA
181	GGCUGAG CUGAUGAGGCGGAAAGGCGGAA AACAGC
184	AGAGGCU CUGAUGAGGCGGAAAGGCGGAA AGGAACA
190	AGGAGAA CUGAUGAGGCGGAAAGGCGGAA AGGCGA
192	GAAGGAG CUGAUGAGGCGGAAAGGCGGAA AGAGGCU
193	GAAGGA CUGAUGAGGCGGAAAGGCGGAA AAGAGGC
195	CAGGAAG CUGAUGAGGCGGAAAGGCGGAA AGAAGAG
198	GAUCAGG CUGAUGAGGCGGAAAGGCGGAA AGGAGAA
199	CGAUCAG CUGAUGAGGCGGAAAGGCGGAA AAGGAGA
205	CUGCCAC CUGAUGAGGCGGAAAGGCGGAA AUCAGGA
226	GGCAGAA CUGAUGAGGCGGAAAGGCGGAA AGCGGGG
228	CAGGCAG CUGAUGAGGCGGAAAGGCGGAA AGAGGGU
229	GCAGGCA CUGAUGAGGCGGAAAGGCGGAA AAGAGCG
243	CACUCCA CUGAUGAGGCGGAAAGGCGGAA AGUGCAG
244	UACUCC CUGAUGAGGCGGAAAGGCGGAA AAGUGCA
253	GGGGGCC CUGAUGAGGCGGAAAGGCGGAA AUCACUC
273	CCUGGGG CUGAUGAGGCGGAAAGGCGGAA ACUCUUC
286	UUAGAGA CUGAUGAGGCGGAAAGGCGGAA AGGUCCC
288	GAUUGA CUGAUGAGGCGGAAAGGCGGAA AGAGGUC
290	CUGAUUA CUGAUGAGGCGGAAAGGCGGAA AGAGAGG
292	GGCUGAU CUGAUGAGGCGGAAAGGCGGAA AGAGAGA
295	GAGGGCU CUGAUGAGGCGGAAAGGCGGAA AUUAGAG
302	UGGGCCA CUGAUGAGGCGGAAAGGCGGAA AGGGCUG

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321	AGAAGAU	CUGADGAGGCGAAAGGCGGAA	ADCUGAC
324	UCGAGAA	CUGADGAGGCGAAAGGCGGAA	ADGADCU
326	GUUOGAG	CUGADGAGGCGAAAGGCGGAA	AGAUGAU
327	GGUUGCA	CUGADGAGGCGAAAGGCGGAA	AAGADGA
329	GGGGUUC	CUGADGAGGCGAAAGGCGGAA	AGAAGAU
352	CAUGGGC	CUGADGAGGCGAAAGGCGGAA	ACAGGCU
361	UUGCUAC	CUGADGAGGCGAAAGGCGGAA	ACAUGGG
364	GGUUGC	CUGADGAGGCGAAAGGCGGAA	ACAACAU
374	UCAGCUU	CUGADGAGGCGAAAGGCGGAA	AGGGUUU
391	GOCACUG	CUGADGAGGCGAAAGGCGGAA	AGCUGCC
421	UGGOCAG	CUGADGAGGCGAAAGGCGGAA	AGGGCAU
449	AGCUGGU	CUGADGAGGCGAAAGGCGGAA	ADCUCUC
468	GOCCUUC	CUGADGAGGCGAAAGGCGGAA	AUGGCAC
480	GADGAGG	CUGADGAGGCGAAAGGCGGAA	ACAGGCC
484	AGUAGAU	CUGADGAGGCGAAAGGCGGAA	AGGUACA
487	GGZAGUA	CUGADGAGGCGAAAGGCGGAA	ADGAGGU
489	CUGGGAG	CUGADGAGGCGAAAGGCGGAA	AGADGAG
492	GAOCUGG	CUGADGAGGCGAAAGGCGGAA	AGUAGAU
499	UGAAGAG	CUGADGAGGCGAAAGGCGGAA	ACCUGGG
502	CCUUGRA	CUGADGAGGCGAAAGGCGGAA	AGGACCU
504	GOCCUUG	CUGADGAGGCGAAAGGCGGAA	AGAGGAC
505	GGCCUUC	CUGADGAGGCGAAAGGCGGAA	AAGAGGA
525	ADGGGUG	CUGADGAGGCGAAAGGCGGAA	AGGGGCA
538	GGGUGAG	CUGADGAGGCGAAAGGCGGAA	AGCACAU
541	UGUGGGU	CUGADGAGGCGAAAGGCGGAA	AGGAGCA
553	UGCGGCU	CUGADGAGGCGAAAGGCGGAA	AUGGUGU
562	AGACGGC	CUGADGAGGCGAAAGGCGGAA	AUGCGGC
568	GGUAGGA	CUGADGAGGCGAAAGGCGGAA	ACGGCGA
570	CUGGUAG	CUGADGAGGCGAAAGGCGGAA	AGAAGGC
573	GGUCUGG	CUGADGAGGCGAAAGGCGGAA	AGGAGAC
586	GGAGGUU	CUGADGAGGCGAAAGGCGGAA	ACCUUGG
592	CAGAGAG	CUGADGAGGCGAAAGGCGGAA	AGGUUGA
595	UGGCAGA	CUGADGAGGCGAAAGGCGGAA	AGGAGGU
597	GADGGCA	CUGADGAGGCGAAAGGCGGAA	AGAGGAG
604	GGCUUCU	CUGADGAGGCGAAAGGCGGAA	AUGGCAG
657	GGGCUCA	CUGADGAGGCGAAAGGCGGAA	ACCAGGG
667	CCAGAUU	CUGADGAGGCGAAAGGCGGAA	AUGGGCU
669	UOCAGA	CUGADGAGGCGAAAGGCGGAA	AGAUGGG
671	CCUCCCA	CUGADGAGGCGAAAGGCGGAA	AUAGADG
682	GCUGGAA	CUGADGAGGCGAAAGGCGGAA	ACCCCTC
684	CAGCUGG	CUGADGAGGCGAAAGGCGGAA	AGACCCC
685	CCAGCUG	CUGADGAGGCGAAAGGCGGAA	AAGACCC
709	CAGCGCU	CUGADGAGGCGAAAGGCGGAA	AGUCGGU
721	GOOGADU	CUGADGAGGCGAAAGGCGGAA	AUCUCAG
725	UOGGGOC	CUGADGAGGCGAAAGGCGGAA	AUUGAUC
735	GUOGAGA	CUGADGAGGCGAAAGGCGGAA	AGUCGGG
737	AAGUCGA	CUGADGAGGCGAAAGGCGGAA	AUAGUCG
739	CAAAGUC	CUGADGAGGCGAAAGGCGGAA	AGAUAGU
744	CUOGGCA	CUGADGAGGCGAAAGGCGGAA	AGUCGAG

745	ACUCCGC	CUGAUGAGGCGAAAGGCGGAA	AAGUCCA
753	CUGCCCA	CUGAUGAGGCGAAAGGCGGAA	ACTCGGC
763	CBAAGUA	CUGAUGAGGCGAAAGGCGGAA	ACCUGCC
765	CCCAAG	CUGAUGAGGCGAAAGGCGGAA	AGACCU
768	GAUCCA	CUGAUGAGGCGAAAGGCGGAA	AGUAGAC
769	UGAUCC	CUGAUGAGGCGAAAGGCGGAA	AAGUAGA
775	GGGCAU	CUGAUGAGGCGAAAGGCGGAA	ADCCCAA
778	ACAGGC	CUGAUGAGGCGAAAGGCGGAA	ADGAUCC
801	AAGGUUG	CUGAUGAGGCGAAAGGCGGAA	AUGUUCG
808	GUUUGG	CUGAUGAGGCGAAAGGCGGAA	AGGUUGG
809	CGUUGG	CUGAUGAGGCGAAAGGCGGAA	AAGGUUG
820	GGCAGG	CUGAUGAGGCGAAAGGCGGAA	AGGCGUU
833	AUAAGG	CUGAUGAGGCGAAAGGCGGAA	AUUGGGG
837	GGUADA	CUGAUGAGGCGAAAGGCGGAA	AGGGAUU
838	GGGUAU	CUGAUGAGGCGAAAGGCGGAA	AAGGGAU
839	GGGUAA	CUGAUGAGGCGAAAGGCGGAA	AAAGGGA
841	AGGGGU	CUGAUGAGGCGAAAGGCGGAA	AUAAGG
842	GAGGGG	CUGAUGAGGCGAAAGGCGGAA	AADAAG
849	UCUGAG	CUGAUGAGGCGAAAGGCGGAA	AGGGGUU
852	GUGUCU	CUGAUGAGGCGAAAGGCGGAA	AGGAGGG
853	GGUGUU	CUGAUGAGGCGAAAGGCGGAA	AAGGAGG
863	AGAGGU	CUGAUGAGGCGAAAGGCGGAA	AGGGUGU
869	GCCAGAA	CUGAUGAGGCGAAAGGCGGAA	AGGUUGA
871	GAGCCAG	CUGAUGAGGCGAAAGGCGGAA	AGAGGUU
872	UGAGCA	CUGAUGAGGCGAAAGGCGGAA	AAGAGGU
878	UCUUUU	CUGAUGAGGCGAAAGGCGGAA	AGCCAGA
890	AGCCCC	CUGAUGAGGCGAAAGGCGGAA	AUUCUCU
898	CGAOCU	CUGAUGAGGCGAAAGGCGGAA	AGCCCC
899	CGAACC	CUGAUGAGGCGAAAGGCGGAA	AAGCCCC
904	GGGUCC	CUGAUGAGGCGAAAGGCGGAA	ACCUUAA
917	AAGUUCU	CUGAUGAGGCGAAAGGCGGAA	AGCUUGG
918	AAAGUUC	CUGAUGAGGCGAAAGGCGGAA	AAGCUUG
924	UUGCUUA	CUGAUGAGGCGAAAGGCGGAA	AGUUCUA
925	GUUGCUU	CUGAUGAGGCGAAAGGCGGAA	AAGUUCU
926	UGUUGCU	CUGAUGAGGCGAAAGGCGGAA	AAAGUUC
945	GGUUCG	CUGAUGAGGCGAAAGGCGGAA	AGUGGUG
946	AGGUUC	CUGAUGAGGCGAAAGGCGGAA	AAGUGGU
959	AUUCUUG	CUGAUGAGGCGAAAGGCGGAA	AUCCAG
960	CAUUCU	CUGAUGAGGCGAAAGGCGGAA	AADCCCA
1001	GAUUCU	CUGAUGAGGCGAAAGGCGGAA	AGUGGUU
1007	CAGUUG	CUGAUGAGGCGAAAGGCGGAA	AUUCUUA
1008	CCAGUU	CUGAUGAGGCGAAAGGCGGAA	AADUCUU
1021	AGUUCU	CUGAUGAGGCGAAAGGCGGAA	AGGCCCC
1029	CCCCAGU	CUGAUGAGGCGAAAGGCGGAA	AGUUCUG
1040	AAAGCUG	CUGAUGAGGCGAAAGGCGGAA	AGGCCCC
1046	GGGAUCA	CUGAUGAGGCGAAAGGCGGAA	AGCUGUA
1047	AGGGADC	CUGAUGAGGCGAAAGGCGGAA	AAGCGGU
1051	UGUCAGG	CUGAUGAGGCGAAAGGCGGAA	AUCAAG
1060	GAUCCA	CUGAUGAGGCGAAAGGCGGAA	ADGUCAG

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1067	GUCUCCA	CUGAUGAGGCGAAAGGCGCGAA	AUUCACG
1085	AGAACCA	CUGAUGAGGCGAAAGGCGCGAA	AGGCUCC
1086	CAGAACC	CUGAUGAGGCGAAAGGCGCGAA	AAGGCUC
1090	UGGOCAG	CUGAUGAGGCGAAAGGCGCGAA	ACCAAAG
1091	CUGGCCA	CUGAUGAGGCGAAAGGCGCGAA	AACCAAA
1113	UCUUCUC	CUGAUGAGGCGAAAGGCGCGAA	AGUCCUG
1124	UCUAGGU	CUGAUGAGGCGAAAGGCGCGAA	AGGUCUU
1129	CAAUUUC	CUGAUGAGGCGAAAGGCGCGAA	AGGUGAG
1135	UUGUGUC	CUGAUGAGGCGAAAGGCGCGAA	AUUCUA
1151	AAGGCCU	CUGAUGAGGCGAAAGGCGCGAA	AGGUGCA
1152	GAAGGCC	CUGAUGAGGCGAAAGGCGCGAA	AAGGUCC
1158	AGAGAGG	CUGAUGAGGCGAAAGGCGCGAA	AGGUCUA
1159	GAGAGAG	CUGAUGAGGCGAAAGGCGCGAA	AAGGCCU
1162	CUGGAGA	CUGAUGAGGCGAAAGGCGCGAA	AGGAGG
1164	AUCUGGA	CUGAUGAGGCGAAAGGCGCGAA	AGAGGAA
1166	ACAUUCG	CUGAUGAGGCGAAAGGCGCGAA	AGAGAGG
1174	GUCUGGA	CUGAUGAGGCGAAAGGCGCGAA	ACAUUCG
1175	AGUCUGG	CUGAUGAGGCGAAAGGCGCGAA	AACAUUC
1176	AAGUCUG	CUGAUGAGGCGAAAGGCGCGAA	AAACAUUC
1183	CUCAAGG	CUGAUGAGGCGAAAGGCGCGAA	AGUCUGG
1184	UCUCAAG	CUGAUGAGGCGAAAGGCGCGAA	AAGUCUG
1187	GUGUCUC	CUGAUGAGGCGAAAGGCGCGAA	AGGAAGU
1208	CCAGGGG	CUGAUGAGGCGAAAGGCGCGAA	AGGCGUG
1224	AUAGAGG	CUGAUGAGGCGAAAGGCGCGAA	AGCUGGC
1228	AUAAUA	CUGAUGAGGCGAAAGGCGCGAA	AGGGAGC
1230	ACAUAAA	CUGAUGAGGCGAAAGGCGCGAA	AGAGGGA
1232	AAACAU	CUGAUGAGGCGAAAGGCGCGAA	AUAGAGG
1233	CAACAU	CUGAUGAGGCGAAAGGCGCGAA	AUAGAG
1234	GCAACA	CUGAUGAGGCGAAAGGCGCGAA	AAAUAGA
1238	AAGUGCA	CUGAUGAGGCGAAAGGCGCGAA	ACAUAAA
1239	CAAGUGC	CUGAUGAGGCGAAAGGCGCGAA	AACAUAA
1245	UAADUAC	CUGAUGAGGCGAAAGGCGCGAA	AGUGCAA
1251	AAUAAAU	CUGAUGAGGCGAAAGGCGCGAA	AUCACAA
1252	UAUUAAA	CUGAUGAGGCGAAAGGCGCGAA	AUUCACA
1254	AAUAAUA	CUGAUGAGGCGAAAGGCGCGAA	AUAUACA
1255	AAAUAAU	CUGAUGAGGCGAAAGGCGCGAA	AAUAUUC
1256	UAUUAAA	CUGAUGAGGCGAAAGGCGCGAA	AAUAUAU
1258	AAUAAAU	CUGAUGAGGCGAAAGGCGCGAA	AUAUAUA
1259	AAAUAAA	CUGAUGAGGCGAAAGGCGCGAA	AAUAUAU
1261	AUAUAUA	CUGAUGAGGCGAAAGGCGCGAA	AUAUAUA
1262	AAUAAAU	CUGAUGAGGCGAAAGGCGCGAA	AAUAUAU
1263	UAUUAAA	CUGAUGAGGCGAAAGGCGCGAA	AAUAUAU
1265	AAUAUAU	CUGAUGAGGCGAAAGGCGCGAA	AUAUAUA
1266	AAUAUAU	CUGAUGAGGCGAAAGGCGCGAA	AAUAUAU
1267	UAUUAAA	CUGAUGAGGCGAAAGGCGCGAA	AAUAUAU
1269	AAUAUAU	CUGAUGAGGCGAAAGGCGCGAA	AUAUAUA
1270	AAAUAAA	CUGAUGAGGCGAAAGGCGCGAA	AAUAUAU
1272	AUAUAUA	CUGAUGAGGCGAAAGGCGCGAA	AUAUAUA
1273	AAUAUAU	CUGAUGAGGCGAAAGGCGCGAA	AAUAUAU

1274	AAATAAA	CUGAUGAGGCGGAAAGGCGCGAA	AAATAAU
1276	GUAAAUA	CUGAUGAGGCGGAAAGGCGCGAA	AUAAAUA
1277	UGUAAAU	CUGAUGAGGCGGAAAGGCGCGAA	AAUAAAU
1278	CUGUAAA	CUGAUGAGGCGGAAAGGCGCGAA	AAATAAA
1280	AUCUGUA	CUGAUGAGGCGGAAAGGCGCGAA	AUAAAUA
1281	CADCUGU	CUGAUGAGGCGGAAAGGCGCGAA	AAUAAAU
1282	UCAUCUG	CUGAUGAGGCGGAAAGGCGCGAA	AAATAAA
1294	AAATAAA	CUGAUGAGGCGGAAAGGCGCGAA	ACAUCCA
1296	CCAAAUA	CUGAUGAGGCGGAAAGGCGCGAA	AUACAUA
1297	CCCAAAU	CUGAUGAGGCGGAAAGGCGCGAA	AAUACAU
1298	UCCCAAA	CUGAUGAGGCGGAAAGGCGCGAA	AAATACA
1300	UCCCCCA	CUGAUGAGGCGGAAAGGCGCGAA	AUAAAUA
1301	GUCUCCC	CUGAUGAGGCGGAAAGGCGCGAA	AAUAAAU
1315	CCACGGA	CUGAUGAGGCGGAAAGGCGCGAA	ACCCCCG
1317	CCCCCAG	CUGAUGAGGCGGAAAGGCGCGAA	AUACCCC
1334	CAGCUCU	CUGAUGAGGCGGAAAGGCGCGAA	ACAUUGG
1345	CUGAGCC	CUGAUGAGGCGGAAAGGCGCGAA	AGGCAGC
1350	CAUGUCU	CUGAUGAGGCGGAAAGGCGCGAA	AGCCAG
1359	CACGGAA	CUGAUGAGGCGGAAAGGCGCGAA	ACAUUGC
1360	UCACGGA	CUGAUGAGGCGGAAAGGCGCGAA	AACAUUU
1361	UUCACGG	CUGAUGAGGCGGAAAGGCGCGAA	AAACAUU
1362	UUUCACG	CUGAUGAGGCGGAAAGGCGCGAA	AAAACAU
1386	AACAGCC	CUGAUGAGGCGGAAAGGCGCGAA	AUUGUUC
1393	ACAUUGG	CUGAUGAGGCGGAAAGGCGCGAA	ACAGCCU
1394	UACAUUG	CUGAUGAGGCGGAAAGGCGCGAA	AACAGCC
1401	AGGGGGC	CUGAUGAGGCGGAAAGGCGCGAA	ACAUUGG
1414	AGGCACA	CUGAUGAGGCGGAAAGGCGCGAA	AGGCCAG
1422	UCAAAAG	CUGAUGAGGCGGAAAGGCGCGAA	AGGCACA
1423	AUCAAAA	CUGAUGAGGCGGAAAGGCGCGAA	AAGGCAC
1425	UAADCAA	CUGAUGAGGCGGAAAGGCGCGAA	AGAAGGC
1426	AUAADCA	CUGAUGAGGCGGAAAGGCGCGAA	AAGAAGG
1427	CAUAADC	CUGAUGAGGCGGAAAGGCGCGAA	AAAGAAG
1431	AAAACAU	CUGAUGAGGCGGAAAGGCGCGAA	AUCAAAA
1432	AAAAACA	CUGAUGAGGCGGAAAGGCGCGAA	AADCAAA
1436	UUUAAA	CUGAUGAGGCGGAAAGGCGCGAA	ACAUAUU
1437	UUUUAAA	CUGAUGAGGCGGAAAGGCGCGAA	AACAUAU
1438	AUUUUAA	CUGAUGAGGCGGAAAGGCGCGAA	AAACAUA
1439	UAUUUUA	CUGAUGAGGCGGAAAGGCGCGAA	AAAACAU
1440	AUAUUUU	CUGAUGAGGCGGAAAGGCGCGAA	AAAAACA
1441	AAUAUUU	CUGAUGAGGCGGAAAGGCGCGAA	AAAAAAC
1446	CAGAUAA	CUGAUGAGGCGGAAAGGCGCGAA	AUUUUAA
1448	AUCAGAU	CUGAUGAGGCGGAAAGGCGCGAA	AUAUUUU
1449	AAUCAGA	CUGAUGAGGCGGAAAGGCGCGAA	AAUAUUU
1451	UUAADCA	CUGAUGAGGCGGAAAGGCGCGAA	AUAUAUU
1456	ACAACUU	CUGAUGAGGCGGAAAGGCGCGAA	AUCAGAU
1457	GACAACU	CUGAUGAGGCGGAAAGGCGCGAA	AADCAGA
1461	UUUAGAC	CUGAUGAGGCGGAAAGGCGCGAA	ACUUAAU
1464	UUGUUUA	CUGAUGAGGCGGAAAGGCGCGAA	ACAACUU
1466	CAUUGUU	CUGAUGAGGCGGAAAGGCGCGAA	AGACAAC

1479	GUCACCA	CUGAUGAGGCGGAAAGGCGGAA	AUCAGCA
1480	GGUCACC	CUGAUGAGGCGGAAAGGCGGAA	AADCAGC
1494	AAUGAGU	CUGAUGAGGCGGAAAGGCGGAA	ACAGUUG
1498	CAGCAAU	CUGAUGAGGCGGAAAGGCGGAA	AGUGACA
1501	CCUCAGC	CUGAUGAGGCGGAAAGGCGGAA	AUGAGUG
1512	GGGAGCA	CUGAUGAGGCGGAAAGGCGGAA	AGGCGUC
1517	CCUGGG	CUGAUGAGGCGGAAAGGCGGAA	AGCAGAG
1528	CAGACAC	CUGAUGAGGCGGAAAGGCGGAA	ACDCCCU
1533	GAUUACA	CUGAUGAGGCGGAAAGGCGGAA	ACACAAC
1537	GGCGAU	CUGAUGAGGCGGAAAGGCGGAA	ACAGACA
1540	GUGGCC	CUGAUGAGGCGGAAAGGCGGAA	AUUACAG
1546	UGAADAG	CUGAUGAGGCGGAAAGGCGGAA	AGGCGGA
1549	CACUGAA	CUGAUGAGGCGGAAAGGCGGAA	AGUAGGC
1551	GCCACTG	CUGAUGAGGCGGAAAGGCGGAA	AUAGUAG
1552	CGCCACU	CUGAUGAGGCGGAAAGGCGGAA	AADAGUA
1566	CAACCUU	CUGAUGAGGCGGAAAGGCGGAA	AUUUCUC
1572	CCUAGC	CUGAUGAGGCGGAAAGGCGGAA	ACCUUUA
1576	CUUUCU	CUGAUGAGGCGGAAAGGCGGAA	AGCAACC
1577	UCUUCC	CUGAUGAGGCGGAAAGGCGGAA	AAGCAAC

Table 25: Mouse TNF- α HH Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
66	UgGAAAU a GcuCCcA	324	GgGUGAU C GGuCCOC
101	GGCAGGU U CUgUcCC	347	GAGAgU u cCCAaaU
101	GGCAGgU u CuGUccC	364	CCUCcCU C UcAUcAG
102	GCAGGUU C UgUcCCU	366	UCcCUU c AUcAGuu
102	gCAGgUU c ugUCCCU	366	UcCCUUC C auCAGuU
106	GUUCGgU c CCUuUCA	369	CCCUcAU C AGuuCUa
110	UgUcCCU u UCAUcA	376	CAGuuCU a UGGCCCA
111	gUCCcCU u CaCUcAC	390	AgACCCU C AcaCUcA
111	guCCCUu u CAUcAc	396	ucaCAcU C AGAUcAU
112	UcCCUuU C ACuAcCU	401	cUCAGAU C AUcUUCU
116	UuUCACU C AcUGgcc	404	AGAUcAU C UUCUCA
137	GCCaCAU C uCCcUCC	406	AUCAUcU U CUcAAA
139	caCAuCU C CCUCcAg	406	AUCAUcU U cUcAAA
177	GCAUGAU C CGcGACG	407	UCAUcCU C UCaaaau
207	AGGCaCU C CCcAaA	409	AUCUUCU C aAAauuC
228	GGGGCuU C CAGAACU	409	AuCuucU c AaAAUUC
228	GGGGCuU c CAGaacU	409	aUcUUCU c AAAauUc
236	CAGaaCU C CAGGCGG	432	AGCCUGU A GCOCAcG
236	CAGaACU c cAGgcGg		
249	GGugCCU a UgUCUcA	444	AcGUcGU A GCAAACC
249	GGUGCCU a UGUcUCA	501	AcGCCCU C CUGGCCA
261	UCAGCCU C UUCUCaU	560	gGgUUGU a CCUguuC
261	UCAgCCU C UUCUcau	560	GGguUGU A CCUgUC
263	AGCCUCU U CUcAUUC	564	UGUAUCU u gUcUACU
263	AgCCUCU U CUcauUC	567	ACCUgU C UACUCC
264	GCCUCU C UCauUCC	569	CUgUCU A CUCCAG
264	gCCUCU C UcauUCC	572	gUCUACU C CCAGGUu
266	CCUCUCU C aUUCUUG	572	GUCUaCU c CCAGguu
269	UUCUCaU U CCUGcUu	572	GuCUaCU C CCAGGUu
270	UCUCaU C CUgcUuG	579	CCAGGU u CUcUUA
276	UCCUGcU u GUGGCAG	580	CCAGguU c uCUUcAa
297	CCACGCU C UUCUGuC	580	CCaGGU c UCuUcaa
299	ACGCUCU U CUGuCUa	582	AGGUUCU C UUCaagg
300	CGCUCU C UGUcUaC	582	AGGUuCU C UUCAAGG
304	CUUCUgU c uAcUGaa	584	GUUCUCU U CAAGGGA
306	UcUGUcU a cUgAAcU	585	UuCCUCU C AAGGGaC
314	CUGaACU U cGgGUG	608	CcCGaCU a CgugCUC
315	UGaACU c GGgGUGA	615	aCgUGcU C CUcAcCC
315	uGaACU c GGgguGa	615	AcGUUCU C CUcAcCC
324	gGUGaU c GgUCCcC	618	UGUUCU C ACCCACa

630	ACACCGU C AGCCGau	940	GUUUAU c cUCAGaG
630	ACACCGU C AgCCgaU	943	UACUccU C AGaGcCc
638	agcCGAU u uGCCaUc	972	UCUazCU u AgAAAGg
643	aUUUGcU a uCUcAuA	972	ucUazCU u AGAaAGg
645	UuGCuaU C UCaUACC	973	CUaACuU A GAAAggG
647	GCuaUCU C aUACCAG	984	AGgGgaU U auGGcuc
663	agAAaGU C AACCUCC	984	AGGGgaU U aUGgCUc
669	UCAACCU C CUUCUCG	985	GGGgauU a uGGcUCa
669	UcAAccU c cUcUCUG	997	UcAGaGU c CAACucU
672	ACCUCCU C UCUGCCg	1010	CugugCU c AGAgCUU
674	CUCCUCU C UGCcGUc	1017	cAGaGU U UcAaCAa
681	cUGCCgU C AagaGcC	1018	AGAgCUU U cAaCAAC
681	CUGCCgU C AAGAGCC	1019	GAgCUU c AaCAACu
681	CUGCCgU C aaGAgcC	1073	UGGGCU c ucAUgCA
734	CCCGGU A UGAGCCC	1096	AAgGAcU C AAAugGG
734	CccUGGU a ugaGCCc	1106	aUGGGcU U uccGAAD
744	AGCCCAU a UAaCUGG	1107	UGGGcUU u ccGAADu
746	CCCAUaU A cCUGGA	1108	GGgCUU c cGaaUUC
759	GAgGAGU C uuCCAGc	1115	CcGAauU C ACUGGag
759	GAGGaGU C UUCACGC	1133	CGAAugU C CAuuCcU
761	GGaGUU U CCAAGUG	1164	gagUGgU c AgGUUGc
762	GaGUUU C CAGCUGG	1180	UcUgUcU c agaAUGA
786	AOCaACU C AGCGCUG	1203	zaGaauU c AGGCCUU
798	CUAGgU C AAUCuGC	1210	cAGGCCU U CCUaCCU
802	GgUCAU C uGCCaA	1211	AGGCCU C CUaCCUu
812	CCCaAgU A cuUaGAC	1214	CCUUCU a cCUuCAG
816	AgUAcuU a GACUUUG	1218	CcuACcU u CaGACUu
821	uUaGACU U UGCgGAG	1218	CCuaCCU U CAGACcu
822	UaGACUU U GCgGAGU	1218	cCuACcU u cAgACCU
830	GCgGAGU C cGGGCAG	1218	CCUaCCU u CAGaccU
840	GGCAGGU C UACUUUG	1219	CuaCCUU C AGACcuu
842	CAGGUCU A CUUUGGa	1219	CuACCUU c agACCUU
842	CAGgucU a CUUugGA	1226	CagACCU U uCCAGAC
842	cagGuCU a CUUUGGA	1226	CAGaccU U UCCAGAC
845	GUUUAU U UGGagUC	1227	agACCUU u CCAGACu
846	UCUAUU U GGagUCA	1227	AGaccUU U CCAGACU
852	UUGGagU C AUUGCuC	1228	GaccUUU C CAGACUc
855	GagUCAU U GCuCGU	1238	gACUCuU c cCUGAGG
887	AUCCaUU c ucUACCC	1262	CAGCCuU C CuCacaG
891	AuuCuCU a CCCaGCC	1283	CCCCccU C uaUUUAU
905	CCcCaCU C UgaCCCC	1283	cCcCCCU C UAUUUAU
905	cCCCaCU c UgaCCCC	1285	cCCCUU A UUUAUAU
905	CcCCACU c uGAcCC	1287	CcuCUAU u UauAuUU
914	GACCCcU U uacUCUG	1287	CCUCUAU U UAUAUUU
915	ACCCCUU u acUCuGA	1288	CUUAUU U AUaUUUG
919	CUUUACU c ugaCCcC	1289	UCUAUU A UaUUUGC
928	GACCCCU u UaUugUC	1293	UUUAUAU U UGCACUU
928	gACCCCU U UAUuguC	1293	uUUaUAU u UGcACuA
932	CCUUUAU U guCUaCU	1294	UUUAUAU U GCACUUA

1300	UUGCACU U aDuADUu	1462	aCCuUGU u GcCuCCU
1303	CACuDaU u AuDuADU	1470	GccuCCU C UUUUGcU
1304	acDuADU A UUCADUA	1472	cuCcCCU U UUGcUUA
1306	UuADUAD U UADUADU	1473	uCcCCU U UGcUUAU
1307	uADUADU U ADUADUU	1474	CcCCU U GcUUAUG
1307	UaDuADU U AuDuADU	1478	UUUGcU U ADGUUa
1308	AGUADUU A UCAUUA	1479	UUUGcU a UGUUAA
1310	UauDuAD U ADUADUU	1479	UUUGcU A UGUUaa
1310	UADUADU U ADUADUU	1484	UUAUGUU U aaaAcAA
1310	UADUADU U ADUADUU	1498	AAAUauU U ADUaAc
1311	ADUADU A UGUADUU	1511	AcccAaU U GUCUAA
1311	ADUADU A UGUADUU	1514	cAaUUGU C UuAAuAA
1311	AuuADU A UuDuADU	1516	aUUGUCU u AAuAAcG
1313	UUAUADU U UADUADU	1529	CgcugAU u UGUgAC
1313	UUAUADU U UADUADU	1529	cGUgAU U UGUgAC
1313	uUAUADU u UauUUAu	1530	gCUgAUU u gGUgacC
1314	UADUADU U ADUADUU	1530	GCUGAUU U GGUgACC
1314	UADUADU U ADUADUU	1563	UgaAcCU c UGcUCCC
1315	ADUADUU A UUCADUA	1563	ugaaCCU C UGUCCC
1317	UADUADU U UADUADU	1568	CUCUGCU C CCCAcGG
1318	ADUADU U ADUADUU	1589	UGaCUGU A ADuGcCC
1319	UUAUADU A UUAUUA	1592	CUGUAAU u GcCCUAC
1326	ADUADUU A UUCADUU	1617	GAGAAAU A AAGaUcG
1328	UADUADU U UADUUGc	1623	UAAAGaU c GCUUaa
1329	ADUADU U ADUUGCu	1633	UUAaaaU a aaAAcC
1330	UUADUU A UUGCu	25	AgGgaCU a gCCagGA
1332	UADUADU U UgCuADU		
1333	ADUADU U gCuADU		
1337	auUUGCU U AuGAuG		
1338	uUUGCU A uGAuGu		
1346	UGAADU A UUAUADU		
1348	AAUGUAD U UAUUUGG		
1349	ADGUADU U AUUUGGa		
1350	UGUADUU A UUGGaA		
1352	uADuADU u UGGaAGG		
1352	UADUADU U UGGaAGg		
1353	ADUADU U GGaAGgC		
1369	GGGUGU C CUGGaGG		
1398	gCUguCU U cAGACAg		
1398	GCUGaCU U cagaCAG		
1412	GACADU U UUCuGUG		
1413	ACADGUU U UCuGUGA		
1414	CAUGUUU U CuGUGAA		
1415	ADGUUUU C uGUGAAA		
1415	ADGUUUU c UgugAaA		
1438	gaGCGU c CCCAccU		
1451	CUGGCU C UCUaCCU		
1453	ggCCUCU C UaCCuUG		

Table 26: Mouse TNF- α Hammerhead Ribozyme Sequences

nt. Position	Mouse HH Ribozyme Sequence
25	UCCUGGC CUGAUGAGGCGGAAAGGCGGAA AGUCUU
66	UGGAGC CUGAUGAGGCGGAAAGGCGGAA AUUUCCA
101	GGACAG CUGAUGAGGCGGAAAGGCGGAA ACCUGCC
101	GGACAG CUGAUGAGGCGGAAAGGCGGAA ACCUGCC
102	AGGACA CUGAUGAGGCGGAAAGGCGGAA AACUCC
102	AGGACA CUGAUGAGGCGGAAAGGCGGAA AACUCC
106	UGAAGG CUGAUGAGGCGGAAAGGCGGAA ACAGAAC
110	UGAGUA CUGAUGAGGCGGAAAGGCGGAA AGGACA
111	GUGAGUG CUGAUGAGGCGGAAAGGCGGAA AAGGAC
111	GUGAGUG CUGAUGAGGCGGAAAGGCGGAA AAGGAC
112	AGUGAU CUGAUGAGGCGGAAAGGCGGAA AAAGGA
116	GGCAGU CUGAUGAGGCGGAAAGGCGGAA AGUGAA
137	GGAGGA CUGAUGAGGCGGAAAGGCGGAA AUGUGCC
139	CUGAGG CUGAUGAGGCGGAAAGGCGGAA AGADUG
177	CGUGGG CUGAUGAGGCGGAAAGGCGGAA AUCADGC
207	UUUGGG CUGAUGAGGCGGAAAGGCGGAA AGUCUU
228	AGUUCUG CUGAUGAGGCGGAAAGGCGGAA AAGCCC
228	AGUUCUG CUGAUGAGGCGGAAAGGCGGAA AAGCCC
236	CGGCUU CUGAUGAGGCGGAAAGGCGGAA AGUUCUG
236	CGGCUU CUGAUGAGGCGGAAAGGCGGAA AGUUCUG
249	UGAGCA CUGAUGAGGCGGAAAGGCGGAA AGGACC
249	UGAGCA CUGAUGAGGCGGAAAGGCGGAA AGGACC
261	AUGAGAA CUGAUGAGGCGGAAAGGCGGAA AGGUGA
261	AUGAGAA CUGAUGAGGCGGAAAGGCGGAA AGGUGA
263	GAUGAG CUGAUGAGGCGGAAAGGCGGAA AGAGGU
263	GAUGAG CUGAUGAGGCGGAAAGGCGGAA AGAGGU
264	GGAUGA CUGAUGAGGCGGAAAGGCGGAA AAGAGG
264	GGAUGA CUGAUGAGGCGGAAAGGCGGAA AAGAGG
266	CAGGAU CUGAUGAGGCGGAAAGGCGGAA AGAAGG
269	AAGCAG CUGAUGAGGCGGAAAGGCGGAA AUGAGAA
270	CAAGCAG CUGAUGAGGCGGAAAGGCGGAA AADGAA
276	CUGCAC CUGAUGAGGCGGAAAGGCGGAA AGCAGG
297	GACAGAA CUGAUGAGGCGGAAAGGCGGAA AGCGUG
299	UAGACAG CUGAUGAGGCGGAAAGGCGGAA AGAGGU
300	GUAGCA CUGAUGAGGCGGAAAGGCGGAA AAGAGG
304	UUCAGUA CUGAUGAGGCGGAAAGGCGGAA ACAGAG
306	AGUUCAG CUGAUGAGGCGGAAAGGCGGAA AGACAG
314	CACCCG CUGAUGAGGCGGAAAGGCGGAA AGUUCAG
315	UACCCC CUGAUGAGGCGGAAAGGCGGAA AAGUUA

315	UCACCC	CUGADGAGGCGGAAGGCGGA	AAGTUC
324	GGGACC	CUGADGAGGCGGAAGGCGGA	AUCACCC
324	GGGACC	CUGADGAGGCGGAAGGCGGA	AUCACCC
347	AUUUGG	CUGADGAGGCGGAAGGCGGA	ACTUCUC
364	CTGAGC	CUGADGAGGCGGAAGGCGGA	AGGGAGG
366	AACUGA	CUGADGAGGCGGAAGGCGGA	AGAGGGA
366	AACUGA	CUGADGAGGCGGAAGGCGGA	AGAGGGA
369	UAGAAC	CUGADGAGGCGGAAGGCGGA	ADGAGAG
376	UGGGCC	CUGADGAGGCGGAAGGCGGA	AGAACCG
390	UGAGGU	CUGADGAGGCGGAAGGCGGA	AGGAGCU
396	ADGAGC	CUGADGAGGCGGAAGGCGGA	AGUGGGA
401	AGAAGU	CUGADGAGGCGGAAGGCGGA	AUCGAG
404	UUGAGA	CUGADGAGGCGGAAGGCGGA	ADGAGCU
406	UUUGAG	CUGADGAGGCGGAAGGCGGA	AGADGAG
406	UUUGAG	CUGADGAGGCGGAAGGCGGA	AGADGAG
407	AUUUGA	CUGADGAGGCGGAAGGCGGA	AAGADGA
409	GAUUUU	CUGADGAGGCGGAAGGCGGA	AGAAGAU
409	GAUUUU	CUGADGAGGCGGAAGGCGGA	AGAAGAU
409	GAUUUU	CUGADGAGGCGGAAGGCGGA	AGAAGAU
432	CGUGGC	CUGADGAGGCGGAAGGCGGA	ACAGGCU
444	GGUUGC	CUGADGAGGCGGAAGGCGGA	AGGAGCU
501	UGGCGC	CUGADGAGGCGGAAGGCGGA	AGGCGCU
560	GACAGG	CUGADGAGGCGGAAGGCGGA	ACAACCC
560	GACAGG	CUGADGAGGCGGAAGGCGGA	ACAACCC
564	AGUAGC	CUGADGAGGCGGAAGGCGGA	AGUAGCA
567	GGGAGU	CUGADGAGGCGGAAGGCGGA	ACAAGCU
569	CGGGAG	CUGADGAGGCGGAAGGCGGA	AGACAGG
572	AACUUG	CUGADGAGGCGGAAGGCGGA	AGUAGAC
572	AACUUG	CUGADGAGGCGGAAGGCGGA	AGUAGAC
572	AACUUG	CUGADGAGGCGGAAGGCGGA	AGUAGAC
579	UGAAGG	CUGADGAGGCGGAAGGCGGA	ACCUUGG
580	UUGAGA	CUGADGAGGCGGAAGGCGGA	AACUUGG
580	UUGAGA	CUGADGAGGCGGAAGGCGGA	AACUUGG
582	CCUUGA	CUGADGAGGCGGAAGGCGGA	AGAAGCU
582	CCUUGA	CUGADGAGGCGGAAGGCGGA	AGAAGCU
584	UCCUUG	CUGADGAGGCGGAAGGCGGA	AGAGAAC
585	GUCUUU	CUGADGAGGCGGAAGGCGGA	AAGAGAA
608	GAGCAG	CUGADGAGGCGGAAGGCGGA	AGUUGGG
615	GGGUGG	CUGADGAGGCGGAAGGCGGA	AGCAGCU
615	GGGUGG	CUGADGAGGCGGAAGGCGGA	AGCAGCU
618	UGUGGU	CUGADGAGGCGGAAGGCGGA	AGGAGCA
630	AUCGGU	CUGADGAGGCGGAAGGCGGA	ACGGUGU
630	AUCGGU	CUGADGAGGCGGAAGGCGGA	ACGGUGU
638	GADAGC	CUGADGAGGCGGAAGGCGGA	ADGGGCU
643	UAGAGA	CUGADGAGGCGGAAGGCGGA	AGCAAAT
645	GGUAGA	CUGADGAGGCGGAAGGCGGA	ADAGCAA
647	CUGGUA	CUGADGAGGCGGAAGGCGGA	AGAUAGC

663	GGAGGUU	CUGADGAGGCOGAAAGGCOGAA	ACUUCUU
669	CAGAGAG	CUGADGAGGCOGAAAGGCOGAA	AGGUUGA
669	CAGAGAG	CUGADGAGGCOGAAAGGCOGAA	AGGUUGA
672	CGGCAGA	CUGADGAGGCOGAAAGGCOGAA	AGGAGGU
674	GAOGCA	CUGADGAGGCOGAAAGGCOGAA	AGAGGAG
681	GGUCUUU	CUGADGAGGCOGAAAGGCOGAA	ACGGCAG
681	GGUCUUU	CUGADGAGGCOGAAAGGCOGAA	ACGGCAG
681	GGUCUUU	CUGADGAGGCOGAAAGGCOGAA	ACGGCAG
734	GGGCUCA	CUGADGAGGCOGAAAGGCOGAA	ACCAGGG
734	GGGCUCA	CUGADGAGGCOGAAAGGCOGAA	ACCAGGG
744	CCAGGUA	CUGADGAGGCOGAAAGGCOGAA	AUGGGCU
746	UCCAGG	CUGADGAGGCOGAAAGGCOGAA	ADADGGG
759	GUUGGAA	CUGADGAGGCOGAAAGGCOGAA	ACUCCDC
759	GUUGGAA	CUGADGAGGCOGAAAGGCOGAA	ACUCCDC
761	CAGCUGG	CUGADGAGGCOGAAAGGCOGAA	AGACCCC
762	CCAGCUG	CUGADGAGGCOGAAAGGCOGAA	AAGACCC
786	CAGCGCU	CUGADGAGGCOGAAAGGCOGAA	AGUUGGU
798	GCAGAUU	CUGADGAGGCOGAAAGGCOGAA	ACUUCAG
802	UUGGGCA	CUGADGAGGCOGAAAGGCOGAA	AUUGACC
812	GUUAAG	CUGADGAGGCOGAAAGGCOGAA	ACUUGGG
816	CAAAGUC	CUGADGAGGCOGAAAGGCOGAA	AAGUACU
821	CUCCGCA	CUGADGAGGCOGAAAGGCOGAA	AGUCUAA
822	ACUCCGC	CUGADGAGGCOGAAAGGCOGAA	AAGUCUA
830	CUCCCGG	CUGADGAGGCOGAAAGGCOGAA	ACUCCGC
840	CAAAGUA	CUGADGAGGCOGAAAGGCOGAA	ACUUGCC
842	UCCAAAG	CUGADGAGGCOGAAAGGCOGAA	AGACCCG
842	UCCAAAG	CUGADGAGGCOGAAAGGCOGAA	AGACCCG
842	UCCAAAG	CUGADGAGGCOGAAAGGCOGAA	AGACCCG
845	GACUCCA	CUGADGAGGCOGAAAGGCOGAA	AGUAGAC
846	UGACUCC	CUGADGAGGCOGAAAGGCOGAA	AAGUAGA
852	GAGCAAU	CUGADGAGGCOGAAAGGCOGAA	ACUCCAA
855	ACAGAGC	CUGADGAGGCOGAAAGGCOGAA	ADGACTC
887	GGGUAGA	CUGADGAGGCOGAAAGGCOGAA	AUUGGAU
891	GGUUGGG	CUGADGAGGCOGAAAGGCOGAA	AGAGAUA
905	GGGGUCA	CUGADGAGGCOGAAAGGCOGAA	AGUGGGG
905	GGGGUCA	CUGADGAGGCOGAAAGGCOGAA	AGUGGGG
905	GGGGUCA	CUGADGAGGCOGAAAGGCOGAA	AGUGGGG
914	CAGAGUA	CUGADGAGGCOGAAAGGCOGAA	AGGGGUC
915	UCAGAGU	CUGADGAGGCOGAAAGGCOGAA	AAGGGGU
919	GGGGUCA	CUGADGAGGCOGAAAGGCOGAA	AGUAAAG
928	GACAAUA	CUGADGAGGCOGAAAGGCOGAA	AGGGGUC
928	GACAAUA	CUGADGAGGCOGAAAGGCOGAA	AGGGGUC
932	AGUAGAC	CUGADGAGGCOGAAAGGCOGAA	AUAAGG
940	CUUGAG	CUGADGAGGCOGAAAGGCOGAA	AGUAGAC
943	GGGCUUU	CUGADGAGGCOGAAAGGCOGAA	AGGAGUA
972	CCUUUCU	CUGADGAGGCOGAAAGGCOGAA	AGUUGA
972	CCUUUCU	CUGADGAGGCOGAAAGGCOGAA	AGUUGA
973	CCUUUC	CUGADGAGGCOGAAAGGCOGAA	AAGUUG
984	GAGCCAU	CUGADGAGGCOGAAAGGCOGAA	AUCCCUU

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NUC 37860

984 GAGCCAU CUGAUGAGGCGGAAAGGCCGAA AUCCCCU
985 UGAGCCA CUGAUGAGGCGGAAAGGCCGAA AADCCCC
997 AGAGUUG CUGAUGAGGCGGAAAGGCCGAA ACUCUGA
1010 AAGCUCU CUGAUGAGGCGGAAAGGCCGAA AGCACAG
1017 UUGUUGA CUGAUGAGGCGGAAAGGCCGAA AGCUCUG
1018 GUGGUGG CUGAUGAGGCGGAAAGGCCGAA AAGCUCU
1019 AGUUGUU CUGAUGAGGCGGAAAGGCCGAA AAAGCUC
1073 UGCADGA CUGAUGAGGCGGAAAGGCCGAA AGGCCCA
1096 CCCAUUU CUGAUGAGGCGGAAAGGCCGAA AGUCCUU
1106 AUUOGGA CUGAUGAGGCGGAAAGGCCGAA AGCCCAU
1107 AAUUCGG CUGAUGAGGCGGAAAGGCCGAA AAGCCCA
1108 GAUUCGG CUGAUGAGGCGGAAAGGCCGAA AAAGCCG
1115 CUCCAGU CUGAUGAGGCGGAAAGGCCGAA AAUUCGG
1133 AGGAADG CUGAUGAGGCGGAAAGGCCGAA ACAUUCG
1164 GCAACCU CUGAUGAGGCGGAAAGGCCGAA ACCACUC
1180 UCAUUCU CUGAUGAGGCGGAAAGGCCGAA AGACAGA
1203 AAGGCCU CUGAUGAGGCGGAAAGGCCGAA AGAUUCU
1210 AGGUAGG CUGAUGAGGCGGAAAGGCCGAA AGGCCUG
1211 AAGGUAG CUGAUGAGGCGGAAAGGCCGAA AAGGCCU
1214 CUGAAGG CUGAUGAGGCGGAAAGGCCGAA AGGAAGG
1218 AGGUCUG CUGAUGAGGCGGAAAGGCCGAA AGGUAGG
1218 AGGUCUG CUGAUGAGGCGGAAAGGCCGAA AGGUAGG
1218 AGGUCUG CUGAUGAGGCGGAAAGGCCGAA AGGUAGG
1218 AGGUCUG CUGAUGAGGCGGAAAGGCCGAA AGGUAGG
1219 AAGGUUU CUGAUGAGGCGGAAAGGCCGAA AAGGUAG
1219 AAGGUUU CUGAUGAGGCGGAAAGGCCGAA AAGGUAG
1226 GUCUGGA CUGAUGAGGCGGAAAGGCCGAA AGGUCUG
1226 GUCUGGA CUGAUGAGGCGGAAAGGCCGAA AGGUCUG
1227 AGUCUGG CUGAUGAGGCGGAAAGGCCGAA AAGGUUU
1227 AGUCUGG CUGAUGAGGCGGAAAGGCCGAA AAGGUUU
1228 GAGUCUG CUGAUGAGGCGGAAAGGCCGAA AAAGGUC
1238 CCUCAGG CUGAUGAGGCGGAAAGGCCGAA AAGAGUC
1262 CUGUGAG CUGAUGAGGCGGAAAGGCCGAA AAGGCTG
1283 AUAATAA CUGAUGAGGCGGAAAGGCCGAA AGGGGGG
1283 AUAATAA CUGAUGAGGCGGAAAGGCCGAA AGGGGGG
1285 AUAATAA CUGAUGAGGCGGAAAGGCCGAA AGAGGGG
1287 AAATAAA CUGAUGAGGCGGAAAGGCCGAA AUAAGAG
1287 AAATAAA CUGAUGAGGCGGAAAGGCCGAA AUAAGAG
1288 CAAATAU CUGAUGAGGCGGAAAGGCCGAA AAUAGAG
1289 GCAATAA CUGAUGAGGCGGAAAGGCCGAA AAATAAG
1293 AAGUGCA CUGAUGAGGCGGAAAGGCCGAA AUAATAA
1293 AAGUGCA CUGAUGAGGCGGAAAGGCCGAA AUAATAA
1294 UAAGUGC CUGAUGAGGCGGAAAGGCCGAA AAATAAA
1300 AAATAAU CUGAUGAGGCGGAAAGGCCGAA AGUGCAA
1303 AAATAAU CUGAUGAGGCGGAAAGGCCGAA AUAAGUG
1304 UAATAAA CUGAUGAGGCGGAAAGGCCGAA AAATAAG
1306 AAATAAA CUGAUGAGGCGGAAAGGCCGAA AUAATAA
1307 AAATAAU CUGAUGAGGCGGAAAGGCCGAA AAATAAA
1307 AAATAAU CUGAUGAGGCGGAAAGGCCGAA AAATAAA

1308	UAAUAA	CUGAUGAGGCGAAAGGCGGAA	AAAUAAU
1310	AAUAAAU	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1310	AAUAAAU	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1310	AAUAAAU	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1311	AAUAAA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1311	AAUAAA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1311	AAUAAA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1313	AUAUAAU	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1313	AUAUAAU	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1313	AUAUAAU	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1314	AAUAAAU	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1314	AAUAAAU	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1315	UAAUAA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1317	AAUAAAU	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1318	AAUAAAU	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1319	UAAUAA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1326	AAUAAAU	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1328	GCAUAAU	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1329	AGCAAAU	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1330	AAGCAA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1332	AUAAGCA	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1333	CAUAAGC	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1337	CAUUCAU	CUGAUGAGGCGAAAGGCGGAA	AGCAAAU
1338	ACAUAUA	CUGAUGAGGCGAAAGGCGGAA	AAGCAA
1346	AAUAAA	CUGAUGAGGCGAAAGGCGGAA	ACAUUCA
1348	CCAAUA	CUGAUGAGGCGAAAGGCGGAA	AUAUAU
1349	UCCAAAU	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1350	UCCAAA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1352	CCUCCA	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1352	CCUCCA	CUGAUGAGGCGAAAGGCGGAA	AUAUAAU
1353	GCCUCC	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1369	CCUCCAG	CUGAUGAGGCGAAAGGCGGAA	ACACCCC
1398	CUGUCUG	CUGAUGAGGCGAAAGGCGGAA	AGACAGC
1398	CUGUCUG	CUGAUGAGGCGAAAGGCGGAA	AGACAGC
1412	CACAGAA	CUGAUGAGGCGAAAGGCGGAA	ACAUGUC
1413	UCAAGA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1414	UUCACAG	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1415	UUUACA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1415	UUUACA	CUGAUGAGGCGAAAGGCGGAA	AAUAAAU
1438	AGGUGGG	CUGAUGAGGCGAAAGGCGGAA	ACAGUUC
1451	AGGUAGA	CUGAUGAGGCGAAAGGCGGAA	AGGCGAG
1453	CAAGGUA	CUGAUGAGGCGAAAGGCGGAA	AGGCGGC
1455	AACAAGG	CUGAUGAGGCGAAAGGCGGAA	AGGAGGG
1462	AGGAGGC	CUGAUGAGGCGAAAGGCGGAA	ACAAGGU
1470	AGCAAAA	CUGAUGAGGCGAAAGGCGGAA	AGGAGGC
1472	UAAGCAA	CUGAUGAGGCGAAAGGCGGAA	AGGAGAG
1473	AUAAGCA	CUGAUGAGGCGAAAGGCGGAA	AAGAGGA
1474	CAUAAGC	CUGAUGAGGCGAAAGGCGGAA	AAAGAGG
1478	UAAACAU	CUGAUGAGGCGAAAGGCGGAA	AGCAAAA

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1479	UUAACA	CUGAUGAGGCGAAAGGCGAA	AAGCAA
1479	UUAACA	CUGAUGAGGCGAAAGGCGAA	AAGCAA
1484	UUGUUU	CUGAUGAGGCGAAAGGCGAA	AACAUA
1498	GUAGAU	CUGAUGAGGCGAAAGGCGAA	AAUAUU
1511	UUAAGAC	CUGAUGAGGCGAAAGGCGAA	AUUGGU
1514	UUAUUA	CUGAUGAGGCGAAAGGCGAA	ACAAUG
1516	CGUUAU	CUGAUGAGGCGAAAGGCGAA	AGACAU
1529	GUACCA	CUGAUGAGGCGAAAGGCGAA	AUCAGG
1529	GUACCA	CUGAUGAGGCGAAAGGCGAA	AUCAGG
1530	GGUCAC	CUGAUGAGGCGAAAGGCGAA	AACAGC
1530	GGUCAC	CUGAUGAGGCGAAAGGCGAA	AACAGC
1563	GGAGCA	CUGAUGAGGCGAAAGGCGAA	AGGUCA
1563	GGAGCA	CUGAUGAGGCGAAAGGCGAA	AGGUCA
1568	CGUGGG	CUGAUGAGGCGAAAGGCGAA	AGCAGG
1589	GGCAAU	CUGAUGAGGCGAAAGGCGAA	ACAGUA
1592	GUAGGC	CUGAUGAGGCGAAAGGCGAA	AUACAG
1617	CGAUUU	CUGAUGAGGCGAAAGGCGAA	AUUUCU
1623	UUUAGC	CUGAUGAGGCGAAAGGCGAA	AUCUUA
1633	GGUUUU	CUGAUGAGGCGAAAGGCGAA	AUUUUA

Table 27: Human TNP- α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
46	AGCCGUGG AGAA GUUUGU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	ACUACU GAC CCACGGCU
54	GAGGUGG AGAA GUUGGU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	ACCCAGG GCU CCACGGUC
185	GGAGAGAA AGAA GAGGAA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UUUUUA GGC UCUUUCUC
201	CUCCACG AGAA GGAAGG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CCUUUUU GAU CGUUUGAG
230	GUCCAGCA AGAA GAAAGG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CUUUUUU GGC UCUUUGAC
234	CAAGUCC AGAA GGCAGA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UUUGUUU GCU GCAUUUUG
254	CCUCUGG AGAA GAUCAC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GUUAUCG GGC CCCAGAGG
296	GGCCAGAG AGAA GAUUGG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CUUAUCA GGC CUUUUUUU
317	AGAGAUU AGAA GACUCC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GCAGUCA GAU CAUUCUUU
387	GCACUUG AGAA GGUUUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AGGGCCA GCU CCAGUUGG
404	AUUUGCC AGAA GUUUGG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CUUAACC GGC GGGCCAAU
453	GCACACC AGAA GGUUUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AUAACCA GCU GGUUGUGC
518	GUUGAGG AGAA GGUUUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CAAGGCU GGC CCUCGAC
554	GGCUAUG AGAA GUUGGU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	ACCAUCA GGC GCUUUGGC
565	UGGUNGGA AGAA GCGAUG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CAUCGGC GUC UCUUAACA
576	UGACCUU AGAA GGUUGG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CCUACCA GAC CAAGGUCA
607	CCUUCUCC AGAA GGUUGG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UCUUCCA GCU GGAAGAGG
704	AGCCUUA AGAA GUCAAC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GUUAACC GAC UCAAGGCU
726	GAUAGUG AGAA GUUUUA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UCAUUGG GGC CGAGUAUC
730	UCAGAUU AGAA GGCAGA ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UCGGGCC GAC UAUUCUGA
824	GGGAUUG AGAA GGGGAG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CUUUUUU GGC CCANUCCC
1042	GGGAUUA AGAA GUAGGC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GCCUACA GCU UUGAUCCC
1168	CUAGAAAC AGAA GGAAGG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	CUUUUCA GAU GUUUCCAG
1178	UCAGAGAA AGAA GGAAGG ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GUUUUCA GAC UAUUUUGA
1202	AUUGGAG AGAA GGCUC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GAUUUCA GGC CUUUUUUU
1220	AUAGAGG AGAA GGUUCC ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	GGAGCCA GCU CCUCUUAU
1284	AUACAUU AGAA GUAAAU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AUUUACA GAU GAUUGUAU
1340	UGAGCCA AGAA GGUUUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AGAGGUU GGC UUUUUUCA
1390	UACUUGG AGAA GGUUUU ACCAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AUAGGUU GCU CCANUUGA

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1452	ACACUUA AGA GAUAU ACCAGAGAAACACACGCTUGUGUACAUUACCUUGUA	AUUAUCU GAU UAGUUGU
1475	GUACACCA AGA GCAUUG ACCAGAGAAACACACGCTUGUGUACAUUACCUUGUA	CAUUGCU GAU UUGUGUAC
1513	CCUUGGG AGA GAGGCC ACCAGAGAAACACACGCTUGUGUACAUUACCUUGUA	GGCUUCU GCU CCCCAGGG
1541	GAUUGUA AGA GAUUAU ACCAGAGAAACACACGCTUGUGUACAUUACCUUGUA	GUUAUCG GGC UACUAUUC

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Table 28: Mouse TNF- α Hairpin Ribozyme Sequences

nt. Position	Hairpin Ribozyme Sequence	Substrate
103	GUAAAGG AGAA GAACTU ACCAGGAAACACACGUGUGUACAUUACUGUA	AGGUUU GUC CUUUUAC
256	UGAGAGAA AGAA GAGACA ACCAGGAAACACACGUGUGUACAUUACUGUA	UGUUUA GGC UUUUUUA
272	CUCCACA AGAA GGAAUG ACCAGGAAACACACGUGUGUACAUUACUGUA	CAUUUU GCU UUUUUUA
301	GUUCAGUA AGAA GAGGAG ACCAGGAAACACACGUGUGUACAUUACUGUA	CUUUUU GUC UUUUUAC
325	CUUUUGG AGAA GAUCAC ACCAGGAAACACACGUGUGUACAUUACUGUA	GUUUUG GUC CUUUUAG
370	GUCCUUG AGAA GUUGAG ACCAGGAAACACACGUGUGUACAUUACUGUA	CUUUUA GCU CUUUUUC
383	GUGUGAG AGAA GGGCCA ACCAGGAAACACACGUGUGUACAUUACUGUA	UGUUUA GAC CUUUUAC
397	AGAGAGG AGAA GAGUUA ACCAGGAAACACACGUGUGUACAUUACUGUA	ACUUUA GCU CAUUUUU
467	GUCAUUC AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	AGAGUA GCU GAGUUUC
546	AUCCAUU AGAA GGUUCC ACCAGGAAACACACGUGUGUACAUUACUGUA	GGUUUA GGC GAUUUUU
549	UACAAUC AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	GUUUUA GCU GUUUUUU
598	GUUUUGG AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	CUUUUU GGC CUUUUAC
603	AGCAAGU AGAA GGUUUG ACCAGGAAACACACGUGUGUACAUUACUGUA	CUUUUU GAC UUUUUUU
631	AGCAUUC AGAA GGUUUG ACCAGGAAACACACGUGUGUACAUUACUGUA	AGUUUA GGC GAUUUUU
634	GAUAGCA AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	GUUUUA GCU UUUUUUC
675	CUUUUAC AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	CUUUUU GGC GUUUUAG
691	GUUUUGG AGAA GGUUUG ACCAGGAAACACACGUGUGUACAUUACUGUA	AGUUUU GGC CUUUUAC
764	CUUUUUC AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	UUUUUA GCU GAGUUAG
803	AGUUUUG AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	UUUUUU GGC UUUUUUU
895	AGUUUUG AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	CUUUUA GGC CUUUUAC
906	GUUUUGG AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	CUUUUU GAC CUUUUAC
920	UUUUUUG AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	UUUUUU GAC CUUUUUU
953	AGCAACA AGAA GGUUUG ACCAGGAAACACACGUGUGUACAUUACUGUA	GUUUUA GUC UUUUUUU
1175	CUUUUUA AGAA GGUUUG ACCAGGAAACACACGUGUGUACAUUACUGUA	UUUUUU GUC UUUUUUU
1220	CUUUUUG AGAA GGUUUG ACCAGGAAACACACGUGUGUACAUUACUGUA	CUUUUA GAC CUUUUAG
1230	AGCAACA AGAA GGUUUG ACCAGGAAACACACGUGUGUACAUUACUGUA	CUUUUA GAC UUUUUUU
1256	GUUUUGG AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	AGUUUA GGC UUUUUAC
1274	UUUUUGG AGAA GUUUUU ACCAGGAAACACACGUGUGUACAUUACUGUA	AGUUUA GGC CUUUUUA

Table 29: Human bcr/abl HH Target Sequence

Sequence ID No.	HH Target Sequence
<u>b2-a2</u> <u>Junction</u>	
20	UGGCGACCA AUA AGGAGAGAGGC
21	GAGAGAGGC CUU CAGGGGCGAGU
22	AAGAGAGGC UUC AGGGGCGAGU

b3-a2
Junction

23	UAGCGAGAG UUC AAAAGGCGGUC
24	UCAAAGGC CUU CAGGGGCGAGU
25	CAAAGGCGC UUC AGGGGCGAGU

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Table 30: Human *bcr-abl* HH Ribozyme Sequences

Sequence ID No.	HH Ribozyme Sequence
26	GGUUCUUCU CUGAUGAGGCGGAAAGGCGGAA AUGAUGGUA
27	ACUGGCGGCG CUGAUGAGGCGGAAAGGCGGAA AGGCGUUCUC
28	UACUGGCGGCU CUGAUGAGGCGGAAAGGCGGAA AAGGCGUUCU
29	GAAGGCUUUU CUGAUGAGGCGGAAAGGCGGAA AACUGGCUA
30	ACUGGCGGCG CUGAUGAGGCGGAAAGGCGGAA AGGCGUUUGA
31	UACUGGCGGCU CUGAUGAGGCGGAAAGGCGGAA AAGGCGUUUG

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Table 31: RSV (1B) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
10	GGCAAAU A AADCAAU	276	AAAAUAU A CUGAADA
14	AAUAAAU C AADUCAG	283	ACUGAAU A CAACACA
18	AAUCAAU U CAGCCAA	295	ACAAAAU A UGGCACU
19	AUCRAAU C AGCCAAC	303	UGGCACU U UCCCUAU
54	CAADGAU A AUAACAC	304	GGCACUU U CCCCUGG
57	UGAUAU A CACCACA	305	GCACUUU C CCGAUGC
77	UGAUGAU C ACAGACA	309	UUUCCCU A UGOCRAU
94	AGACCGU U GUCACUU	317	UGOCRAU A UUCACCA
97	CCGUUGU C ACUUGAG	319	CCRAUAU U CAUCRAU
101	UGOCACU U GAGACCA	320	CAARAUA C AUCAAUC
110	AGACCAU A AUAACAU	323	UAUCCAU C AADCAUG
113	CCRAUAU A ACACAC	327	CAUCRAU C AUGAUGG
118	AUAACAU C ACUAAOC	337	GAGGGGU U CUAGAA
122	CAUCACU A ACCAGAG	338	AUGGGUU C UUAAGAU
134	GAGACAU C AUAACAC	340	GGGUUCU U AGAUGGC
137	ACACCAU A ACACACA	341	GGUCCUU A GAUUGCA
148	CACAAAU U UAUAAC	350	AAGCCAU U GSCAUUA
149	ACRAAUU U AUAACU	356	UUGGCAU U AAGCCUA
150	CAAAUUU A UAUAUU	357	UGGCAUU A AGCCUAC
152	AAUUUAU A UACUUGA	363	UAAGCCU A CAAAGCA
154	UUUAUAU A CUUGADA	372	AAAGCAU A CUCCCAU
157	AUAUAU U GAUAUAU	375	GCUAUAU C CCAUAUA
161	ACUUGAU A AADCAUG	380	CCCCCAU A AUAUAUA
165	GAUAUAU C AUGAUGG	383	CCAUUAU A UACAAGU
176	AAGCAU A GUGAGAA	385	AUAUAUA A CAAGUAU
188	GAAAAU U GAUGAAA	391	UACAAGU A UGAUCCG
208	GCCACAU U UACAUOC	396	GUAUGAU C UCAAUCC
209	CCACAUU U ACAUOCC	398	AUGAUUU C AADCCAU
210	CACAUUU A CAUUCUU	402	UCUCAU C CAUAAAU
214	UUUACAU U CUGGGUC	406	AAGCCAU A AAUUUCA
215	UUACAUU C CUGGUCA	410	CAGAAAU U UCAACAC
221	UCCUGGU C AACUAGG	411	AUAUAUU U CAACACA
226	GUCACU A UGAUAGG	412	UAUAUUU C AACACAA
239	UGAAACU A UUAACAA	421	ACACAAU A UUCACAC
241	AAACUAU U ACACAAA	423	ACAAUAU U CACACAA
242	AACUAUU A CACAAAG	424	CAADAUU C ACACAAU
251	ACAAAGU A GGAAGCA	432	ACACAAU C UAAAACA
261	AAGCACU A AAUAUAA	434	ACAAUCU A AAACAAC
265	ACUAAAU A UAAAAAA	446	AACACAU C UAUGCAU
267	UAAUAU A AAAAUA	448	CAACUUU A UGAUUA
274	AAAAAAU A UACUGAA	454	UAUGCAU A ACUAUAC

458	CAUACU A UACUCCA
460	UAACUUA A CUCCADA
463	CUAUAU C CAUAGUC
467	ACUCCAU A GUCCAGA
470	CCAUAU C CAGAUCC
489	UGAAAU U AUAUAU
490	GAAAUU A UAGUAU
492	AAAUUA A GUUAUU
495	UAUAU A AUUAUA

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Table 32: RSV (1B) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AUUGADU CUGADGAGGCGGAAAGGCGCGAA AUUGGCC
14	CUGAADU CUGADGAGGCGGAAAGGCGCGAA AUUUAUU
18	UUGGCTG CUGADGAGGCGGAAAGGCGCGAA AUUGAUU
19	GUUGGCU CUGADGAGGCGGAAAGGCGCGAA AAUUGAU
54	GGUGUAU CUGADGAGGCGGAAAGGCGCGAA AUCAUUG
57	UGUGGUG CUGADGAGGCGGAAAGGCGCGAA AUUAUCA
77	UGUCUGU CUGADGAGGCGGAAAGGCGCGAA AUCAUCA
94	AAGUCAC CUGADGAGGCGGAAAGGCGCGAA ACGGUCU
97	CUCAGU CUGADGAGGCGGAAAGGCGCGAA ACAACGG
101	UGGUCUC CUGADGAGGCGGAAAGGCGCGAA AGUGACA
110	AUGUUAU CUGADGAGGCGGAAAGGCGCGAA AUGGUCU
113	GUGADGU CUGADGAGGCGGAAAGGCGCGAA AUUAUGG
118	GGUAGU CUGADGAGGCGGAAAGGCGCGAA AUGUUAU
122	CUCUGGU CUGADGAGGCGGAAAGGCGCGAA AGUGADG
134	GUGUUAU CUGADGAGGCGGAAAGGCGCGAA AUGUCUC
137	UGUGUGU CUGADGAGGCGGAAAGGCGCGAA ADGADGU
148	GUADAUA CUGADGAGGCGGAAAGGCGCGAA AUUGUGG
149	AGUAUAU CUGADGAGGCGGAAAGGCGCGAA AAUUGCU
150	AAGUAUA CUGADGAGGCGGAAAGGCGCGAA AAUUAUG
152	UCAAGUA CUGADGAGGCGGAAAGGCGCGAA AUAAAUU
154	UAUCAAG CUGADGAGGCGGAAAGGCGCGAA AUAUAAA
157	AUUUAUC CUGADGAGGCGGAAAGGCGCGAA AGUAUAU
161	CAUGAUU CUGADGAGGCGGAAAGGCGCGAA AUCAAGU
165	CAUUCAU CUGADGAGGCGGAAAGGCGCGAA AUUAUUC
176	UUCUCAC CUGADGAGGCGGAAAGGCGCGAA ADGCAUU
188	UUUCAUC CUGADGAGGCGGAAAGGCGCGAA AGUUUUC
208	GAUGUA CUGADGAGGCGGAAAGGCGCGAA ADGUGGC
209	GGAADGU CUGADGAGGCGGAAAGGCGCGAA AADGUGG
210	AGGAADG CUGADGAGGCGGAAAGGCGCGAA AAUUGUG
214	GACCAGG CUGADGAGGCGGAAAGGCGCGAA AUGUAAA
215	UGACCAG CUGADGAGGCGGAAAGGCGCGAA AADGUAA
221	CAUGGUU CUGADGAGGCGGAAAGGCGCGAA ACCAGGA
226	CAUUUCA CUGADGAGGCGGAAAGGCGCGAA AGUUGAC
239	UGUGUA CUGADGAGGCGGAAAGGCGCGAA AGUUUCA
241	UUUGUGU CUGADGAGGCGGAAAGGCGCGAA AUAUUUU
242	CUUUGUG CUGADGAGGCGGAAAGGCGCGAA AAUAGUU
251	UGCUUCC CUGADGAGGCGGAAAGGCGCGAA ACUUUGU
261	UUUAUAU CUGADGAGGCGGAAAGGCGCGAA AGUGCUU
265	UUUUUUA CUGADGAGGCGGAAAGGCGCGAA AUUUAGU
267	UAUUUUU CUGADGAGGCGGAAAGGCGCGAA AUUAUUA
274	UUCAGUA CUGADGAGGCGGAAAGGCGCGAA AUUUUUU
276	UAUUCAG CUGADGAGGCGGAAAGGCGCGAA AUUAUUU

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283	UGUGUG	CUGAUGAGGCGGAAAGGCGGAA	AUUCAGU
295	AGUGCCA	CUGAUGAGGCGGAAAGGCGGAA	AUUUGU
303	AUAGGGA	CUGAUGAGGCGGAAAGGCGGAA	AGUGCCA
304	CADAGGG	CUGAUGAGGCGGAAAGGCGGAA	AAGGCGC
305	GCA DAGG	CUGAUGAGGCGGAAAGGCGGAA	AAAGUGC
309	AUUGGCA	CUGAUGAGGCGGAAAGGCGGAA	AGGGA AA
317	UGAUGAA	CUGAUGAGGCGGAAAGGCGGAA	AUUGGCA
319	AUUGAUG	CUGAUGAGGCGGAAAGGCGGAA	AUAUUGG
320	GAUUGAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUUGG
323	CAUGAUU	CUGAUGAGGCGGAAAGGCGGAA	AUGAUAU
327	CCAUCAU	CUGAUGAGGCGGAAAGGCGGAA	AUUGAUG
337	UUCUAAG	CUGAUGAGGCGGAAAGGCGGAA	ACCCADC
338	AUUCUAA	CUGAUGAGGCGGAAAGGCGGAA	AACCCAU
340	GCAUUCU	CUGAUGAGGCGGAAAGGCGGAA	AGAACCC
341	UGCAUUC	CUGAUGAGGCGGAAAGGCGGAA	AAGAACCC
350	UAUUGCC	CUGAUGAGGCGGAAAGGCGGAA	AUGCAUU
356	UAGGCUU	CUGAUGAGGCGGAAAGGCGGAA	AUGCCAA
357	GUAGGCU	CUGAUGAGGCGGAAAGGCGGAA	AAGGCCA
363	UGCUUUG	CUGAUGAGGCGGAAAGGCGGAA	AGGCUUA
372	AUGGGAG	CUGAUGAGGCGGAAAGGCGGAA	AUGCUUU
375	AUAUUGG	CUGAUGAGGCGGAAAGGCGGAA	AGUAUUGC
380	UGUAUAU	CUGAUGAGGCGGAAAGGCGGAA	AUGGGAG
383	ACUUGUA	CUGAUGAGGCGGAAAGGCGGAA	AUAUUGG
385	AUAUCUG	CUGAUGAGGCGGAAAGGCGGAA	AUAUAUU
391	GAGAUCA	CUGAUGAGGCGGAAAGGCGGAA	ACUUGUA
396	GCAUUGA	CUGAUGAGGCGGAAAGGCGGAA	AUCAUAC
398	AUGGAUU	CUGAUGAGGCGGAAAGGCGGAA	AGAUCAU
402	AUUUAUG	CUGAUGAGGCGGAAAGGCGGAA	AUGAGA
406	UGAAAUU	CUGAUGAGGCGGAAAGGCGGAA	AUGGAUU
410	GUGUUGA	CUGAUGAGGCGGAAAGGCGGAA	AUUUAUG
411	UGUGUG	CUGAUGAGGCGGAAAGGCGGAA	AUUUAUU
412	UUGUGUU	CUGAUGAGGCGGAAAGGCGGAA	AAAUUA
421	GUGUGAA	CUGAUGAGGCGGAAAGGCGGAA	AUGUGUU
423	UUGUGUG	CUGAUGAGGCGGAAAGGCGGAA	AUAUUGU
424	AUUGUGU	CUGAUGAGGCGGAAAGGCGGAA	AAUAUUG
432	UGUUUA	CUGAUGAGGCGGAAAGGCGGAA	AUGUGUU
434	GUUGUUU	CUGAUGAGGCGGAAAGGCGGAA	AGAUUGU
446	AUGCAUA	CUGAUGAGGCGGAAAGGCGGAA	AGUUGUU
448	UUAUGCA	CUGAUGAGGCGGAAAGGCGGAA	AGAGUUG
454	GUUAUGU	CUGAUGAGGCGGAAAGGCGGAA	AUGCAUA
458	UGGAGUA	CUGAUGAGGCGGAAAGGCGGAA	AGUAUUG
460	UAUGGAG	CUGAUGAGGCGGAAAGGCGGAA	AUAUAUA
463	GACUAUG	CUGAUGAGGCGGAAAGGCGGAA	AGUAUUG
467	UCUGGAC	CUGAUGAGGCGGAAAGGCGGAA	AUGGAGU
470	CCAUCUG	CUGAUGAGGCGGAAAGGCGGAA	ACUAUUG
489	UUAUAUU	CUGAUGAGGCGGAAAGGCGGAA	AUUUUA
490	AUAUAUA	CUGAUGAGGCGGAAAGGCGGAA	AAUUUA
492	AAAUUAU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAUU
495	UUUAUAU	CUGAUGAGGCGGAAAGGCGGAA	ACUAUAU

Table 33 : RSV (1C) HH target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
10	GGCAAAU A AGAAUUU	165	UACAUUU A ACTAACG
16	UAAGAAU U UGAAAG	169	UUUAAU A ACGCUU
17	AAGAAU U GAUAAGU	175	UACGGU U UGGCUA
21	AUUUGAU A AGUACCA	176	AACGGU U GGUUAG
25	GAUAAGU A CCACUA	181	UUUGGU A AGGCAU
31	UACCAU U AAUUUA	192	CAGUGU A CAUCAA
32	ACCACU A AAUUUA	196	GADACAU A CAUCAA
36	CUUAAU U UAACUCC	201	ADACAU C AAUUUA
37	UUAAAU U AACUCCC	206	AUCAAU U GAUUGG
38	UAAAUU A ACUCCU	216	AUGGCAU U GUGUUG
42	UUUAAU C CUUGGU	221	AUUGGU U UGUGAU
46	ACUCCU U GGUAGA	222	UUGUGU U GUGCAU
50	CUUGGU U AGAGUG	231	UGCAU U AUUCAA
51	CUUGGU A GAGUUG	232	GCAUGU A UUAUAG
67	CAGCAU U CAUUGAG	234	AUGUUAU U ACAUGU
68	AGCAAU C AUUGAU	235	UGUUAU A CAUGU
71	AUUCAU U GAGUAG	241	UACAAGU A GUGUAU
76	AUUGAU A UGAUAA	247	UAGUGU A UUGUCC
81	GUUGAU A AAAGUA	249	GUGUAU U UGUUUA
87	UAAAGU U AGAUUAC	250	UGAUU U GUUUA
88	AAAGU A GAUUA	256	UUGCCU A AUUAUA
92	GUUGAU U ACAAAU	259	CCUUAU A AUUAUA
93	UUAGAU A CAAAUU	262	UAUAU A AUUAU
100	ACAAAU U UGUUGA	265	UAUAU A UUGUAG
101	CAAAAU U GUUGAC	267	AUAUAU U GUAGUA
104	AUUUGU U UGCAAU	270	AUAUUGU A GUAAAU
105	AUUUGU U GACUAG	273	UUGUAGU A AAUCCA
120	AUGAAGU A GCAUUG	278	GUAAAU C CAUUUC
125	GUAGAU U GUUAAA	283	AUCCAU U UCACAAC
128	GCAUUGU U AAUAUA	284	UCCAUU U CACAACA
129	CAUUGU A AAUAUA	285	CCAUU C ACAACA
135	UAAAAU A ACAUGU	300	UGGCAU A CUACAA
143	ACAUGU A UACUGAU	303	CAGUACU A CAAAUU
145	AUGGUU A CUUAUA	316	UGGAGU U AUUAU
151	UACUGAU A AAUAU	317	GGAGGU U UAUAUG
155	GAUAU U AUUAU	319	AGGUUAU A UAUGGA
156	AUAUAU A AUUAU	321	GUUAU A UGGGAA
159	AUAUAU A CAUUA	338	AUGGAU U AACCAU
163	AUAUAU U UAUAUA	339	UGGAU A ACACAU
164	AUAUAU U AUAUA	346	AACACU U CUUUA

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350 CAUUGCU C UCAACCU
 352 UUGCUCU C AACCUAA
 358 UCAACCU A AUGGUCU
 364 UAAUGGU C UACUAGA
 366 AUGGUCU A CUAAGUG
 369 GUUUAU A GAUGACA
 379 UGACAAU U GUGAAAU
 387 GUGAAAU U AAADUCU
 388 UGAAAU A AAUCCUC
 392 AUUAAAU U CUCCAAA
 393 UUAAAAU C UCCAAAA
 395 AAADUCU C CAAAAA
 405 AAAAAGU A AGUGAUU
 412 AAGUGAU U CAACAAU
 413 AGUGAUU C AACAAUG
 427 GAUCAAU U AUUAGAA
 428 ACCAAAU A UAUGAAU
 430 CAUUUAU A UGAADCA
 436 UAUGAAU C AAUUAUC
 440 AUCAAU U AUUUGAA
 441 AUCAAU A UCUGAUU
 443 CAUUUAU C UGAUUUA
 449 UCUGAUU U ACUUGGA
 450 CUUGAAU A CUUGGAU
 453 AAUUAU U GGAUUUG
 458 CUUGGAU U UGADCUU
 459 UUGGAUU U GADCUUA
 463 AUUGAUU C UUAUCCU
 465 UUGADCU U AAUCCAU
 466 UGADCUU A AUCCAUU
 469 UCUUAU C CAUAAAU
 473 AAUCCAU A AAUUAUA
 477 CAUAAAU U AAUUAUA
 478 AAUUAU A UAUAUA
 480 AAUUAU A AUUAUA
 483 UUAUAU U AAUAUA
 484 UAUAUA A AUUAUA
 487 AAUUAU A UCAACUA
 489 UUAUAU C AACUAGC
 494 AUCAAU A GCAAUUC
 501 AGCAAU C AAGUCA
 507 UCAAUU C ACUAACA
 511 UGUCAU A ACACCAU
 519 ACACCAU U AGUUAU
 520 CACCAU A GUUAUA
 523 CAUUAU U AAUAUA
 524 AUUAU A AUUAUA

Table 34: RSV (1C) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
10	AAAUUCU CUGAUGAGGCGAAAGGCGGAA AUUGGOC
16	CUUADCA CUGAUGAGGCGAAAGGCGGAA AUGGUCU
17	ACUUDAC CUGAUGAGGCGAAAGGCGGAA AUUUCUU
21	UGGUACU CUGAUGAGGCGAAAGGCGGAA AUCAAUU
25	UAAGUGG CUGAUGAGGCGAAAGGCGGAA ACUUDAC
31	UAAAUUU CUGAUGAGGCGAAAGGCGGAA AGGUGUA
32	UUAAAUU CUGAUGAGGCGAAAGGCGGAA AAGUGGU
36	GGAGUUA CUGAUGAGGCGAAAGGCGGAA AUUUAAG
37	GGGAGUU CUGAUGAGGCGAAAGGCGGAA AADUUAA
38	AGGGAGU CUGAUGAGGCGAAAGGCGGAA AAADUUA
42	ACCAAGG CUGAUGAGGCGAAAGGCGGAA AGUUAAA
46	UCUAAAC CUGAUGAGGCGAAAGGCGGAA AGGGAGU
50	CADUCUU CUGAUGAGGCGAAAGGCGGAA ACCAAGG
51	CCAUUCU CUGAUGAGGCGAAAGGCGGAA AACCAAG
67	CUCAAUG CUGAUGAGGCGAAAGGCGGAA AUUGGCG
68	ACUCAAU CUGAUGAGGCGAAAGGCGGAA AUGGCUU
71	CAUACUC CUGAUGAGGCGAAAGGCGGAA AUGGAUU
76	UUUAUCA CUGAUGAGGCGAAAGGCGGAA ACUCAAU
81	UAACUUU CUGAUGAGGCGAAAGGCGGAA AUCAUAC
87	GUAAUCU CUGAUGAGGCGAAAGGCGGAA ACUUUUA
88	UGUAUUC CUGAUGAGGCGAAAGGCGGAA AACUUUU
92	AUUUUGU CUGAUGAGGCGAAAGGCGGAA AUUUAAC
93	AAUUUGU CUGAUGAGGCGAAAGGCGGAA AADCUAA
100	UCAAAAC CUGAUGAGGCGAAAGGCGGAA AUUUUGU
101	GUCAAAC CUGAUGAGGCGAAAGGCGGAA AADUUUG
104	AUUGUCA CUGAUGAGGCGAAAGGCGGAA ACUAAUU
105	CADUGUC CUGAUGAGGCGAAAGGCGGAA AACAAAU
120	ACAAUGC CUGAUGAGGCGAAAGGCGGAA ACUUAUU
125	UUUAUAC CUGAUGAGGCGAAAGGCGGAA AUGCUAC
128	UAUUUUU CUGAUGAGGCGAAAGGCGGAA ACAUUGC
129	UUAUUUU CUGAUGAGGCGAAAGGCGGAA AACAAUG
135	AGCAUGU CUGAUGAGGCGAAAGGCGGAA AUUUUUA
143	AUCAGUA CUGAUGAGGCGAAAGGCGGAA AGCAUGU
145	UUUUCAG CUGAUGAGGCGAAAGGCGGAA AUGACAU
151	AUUAAUU CUGAUGAGGCGAAAGGCGGAA AUCAGUA
155	AUGUAUU CUGAUGAGGCGAAAGGCGGAA AUUUUAC
156	AAUGUAU CUGAUGAGGCGAAAGGCGGAA AADUUUU
159	UUAAADG CUGAUGAGGCGAAAGGCGGAA AUUAAUU
163	UUAGUUA CUGAUGAGGCGAAAGGCGGAA AUGUAUU
164	GUUAGUU CUGAUGAGGCGAAAGGCGGAA AADGUUU
165	CGUUAUU CUGAUGAGGCGAAAGGCGGAA AAUUGUA

169	AAAGGUU	CUGAUGAGGCGGAAAGGCGGAA	AGUUAUA
175	UURAGCCA	CUGAUGAGGCGGAAAGGCGGAA	AGGUGUA
176	CUUAGCC	CUGAUGAGGCGGAAAGGCGGAA	AAGGUGU
181	ACUGCCU	CUGAUGAGGCGGAAAGGCGGAA	AGCCRAA
192	UUGUAUG	CUGAUGAGGCGGAAAGGCGGAA	AUCACUG
196	UUGAUUG	CUGAUGAGGCGGAAAGGCGGAA	AUGUADC
201	UCAAUUU	CUGAUGAGGCGGAAAGGCGGAA	AUUGUAU
206	GCCAUUC	CUGAUGAGGCGGAAAGGCGGAA	AUUUGAU
216	CAACAC	CUGAUGAGGCGGAAAGGCGGAA	AUGCCAU
221	AUGCACA	CUGAUGAGGCGGAAAGGCGGAA	ACACAAU
222	CADGCAC	CUGAUGAGGCGGAAAGGCGGAA	AACACAA
231	UUGUAUU	CUGAUGAGGCGGAAAGGCGGAA	ACADGCA
232	CUUGTAA	CUGAUGAGGCGGAAAGGCGGAA	AACADGC
234	UACUUGU	CUGAUGAGGCGGAAAGGCGGAA	AUAACAU
235	CUACUUG	CUGAUGAGGCGGAAAGGCGGAA	AUAACA
241	AUAUCAC	CUGAUGAGGCGGAAAGGCGGAA	ACUUGUA
247	GGGCAA	CUGAUGAGGCGGAAAGGCGGAA	AUCACUA
249	UAGGCA	CUGAUGAGGCGGAAAGGCGGAA	AUAUCAC
250	UUAGGCG	CUGAUGAGGCGGAAAGGCGGAA	AUAUCA
256	UUAUUUU	CUGAUGAGGCGGAAAGGCGGAA	AGGCGAA
259	AUAUUUU	CUGAUGAGGCGGAAAGGCGGAA	AUAUGGG
262	ACAUAUU	CUGAUGAGGCGGAAAGGCGGAA	AUAUUA
265	ACUACAA	CUGAUGAGGCGGAAAGGCGGAA	AUAUAUA
267	UUAUUC	CUGAUGAGGCGGAAAGGCGGAA	AUAUAUU
270	AUUUAC	CUGAUGAGGCGGAAAGGCGGAA	ACAUAUU
273	UGGAUUU	CUGAUGAGGCGGAAAGGCGGAA	ACUACAA
278	GAUAUUG	CUGAUGAGGCGGAAAGGCGGAA	AUUUAC
283	GUUGUGA	CUGAUGAGGCGGAAAGGCGGAA	AUUGGAU
284	UGUUGUG	CUGAUGAGGCGGAAAGGCGGAA	AUUUGGA
285	UUGUUGU	CUGAUGAGGCGGAAAGGCGGAA	AAAUUGG
300	UUGUGAG	CUGAUGAGGCGGAAAGGCGGAA	ACUGGCA
303	CAUUUUG	CUGAUGAGGCGGAAAGGCGGAA	AGUACUG
316	CADAUAU	CUGAUGAGGCGGAAAGGCGGAA	ACCCCCA
317	CCAUADA	CUGAUGAGGCGGAAAGGCGGAA	AACCCCC
319	UCCCAUA	CUGAUGAGGCGGAAAGGCGGAA	AUAACCU
321	UUUCCA	CUGAUGAGGCGGAAAGGCGGAA	AUAUAAC
338	AUGUGUU	CUGAUGAGGCGGAAAGGCGGAA	AUUCCAU
339	AADGUGU	CUGAUGAGGCGGAAAGGCGGAA	AUUCCA
346	UGAGAGC	CUGAUGAGGCGGAAAGGCGGAA	AUGUGUU
350	AGGUUGA	CUGAUGAGGCGGAAAGGCGGAA	AGCAUUG
352	UUAAGGU	CUGAUGAGGCGGAAAGGCGGAA	AGAGCAA
358	AGAUAU	CUGAUGAGGCGGAAAGGCGGAA	AGGUUGA
364	UCUAGUA	CUGAUGAGGCGGAAAGGCGGAA	ACCAUAU
366	CAUCUAG	CUGAUGAGGCGGAAAGGCGGAA	AGACCAU
369	UGUCAUC	CUGAUGAGGCGGAAAGGCGGAA	AGUAGAC
379	AUUUCAC	CUGAUGAGGCGGAAAGGCGGAA	AUUUCA
387	AGAUUU	CUGAUGAGGCGGAAAGGCGGAA	AUUUCAC
388	GAGAUUU	CUGAUGAGGCGGAAAGGCGGAA	AUUUCA
392	UUUGGAG	CUGAUGAGGCGGAAAGGCGGAA	AUUUAUU

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393	UUUUGGA	CUGAUGAGGCGGAAAGGCGGAA	AUUUUA
395	UUUUUG	CUGAUGAGGCGGAAAGGCGGAA	AGAUUU
405	AADCACU	CUGAUGAGGCGGAAAGGCGGAA	AGUUUU
412	AUUGUUG	CUGAUGAGGCGGAAAGGCGGAA	AUCACUU
413	CAUGUUG	CUGAUGAGGCGGAAAGGCGGAA	AADCACU
427	UUCAUAU	CUGAUGAGGCGGAAAGGCGGAA	AUUGGUC
428	AUUCADA	CUGAUGAGGCGGAAAGGCGGAA	AUUUGGU
430	UGAUUCA	CUGAUGAGGCGGAAAGGCGGAA	AUAADUG
436	GAAUAUU	CUGAUGAGGCGGAAAGGCGGAA	AUUCADA
440	UUCAGAU	CUGAUGAGGCGGAAAGGCGGAA	AUUGAUU
441	AUUCAGA	CUGAUGAGGCGGAAAGGCGGAA	AUUUGAU
443	UUAUUCA	CUGAUGAGGCGGAAAGGCGGAA	AUAADUG
449	UCCAAGU	CUGAUGAGGCGGAAAGGCGGAA	AUUCAGA
450	AUCCAAG	CUGAUGAGGCGGAAAGGCGGAA	AUUCAG
453	CRAAUCC	CUGAUGAGGCGGAAAGGCGGAA	AGUAUU
458	AAGAUCA	CUGAUGAGGCGGAAAGGCGGAA	AUCCAAG
459	UAAGAUU	CUGAUGAGGCGGAAAGGCGGAA	AUCCAAG
463	GGAUUA	CUGAUGAGGCGGAAAGGCGGAA	AUCAAUU
465	AUGGAUU	CUGAUGAGGCGGAAAGGCGGAA	AGAUCAA
466	UADGGAU	CUGAUGAGGCGGAAAGGCGGAA	AAGAUCA
469	AUUUAUG	CUGAUGAGGCGGAAAGGCGGAA	AUUAAGA
473	UAUAUUU	CUGAUGAGGCGGAAAGGCGGAA	AUGGAUU
477	UAUUUAU	CUGAUGAGGCGGAAAGGCGGAA	AUUUAUG
478	UUAUUUA	CUGAUGAGGCGGAAAGGCGGAA	AUUUAUU
480	UAUUUAU	CUGAUGAGGCGGAAAGGCGGAA	AUAADUU
483	UGAUUAU	CUGAUGAGGCGGAAAGGCGGAA	AUUUAUA
484	UUGAUUU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAUA
487	UAGUUGA	CUGAUGAGGCGGAAAGGCGGAA	AUAUUUU
489	GCUAGUU	CUGAUGAGGCGGAAAGGCGGAA	AUAUUUA
494	GAAUUGC	CUGAUGAGGCGGAAAGGCGGAA	AGUUGAU
501	UGACAUU	CUGAUGAGGCGGAAAGGCGGAA	AUUUGGU
507	UGUUAUG	CUGAUGAGGCGGAAAGGCGGAA	ACAUUGA
511	AUGGUGU	CUGAUGAGGCGGAAAGGCGGAA	AGUGACA
519	AUUAACU	CUGAUGAGGCGGAAAGGCGGAA	AUGGUGU
520	UAUUUAC	CUGAUGAGGCGGAAAGGCGGAA	AUUUGGU
523	UUUAUUU	CUGAUGAGGCGGAAAGGCGGAA	ACUAUUG
524	UUUAUUU	CUGAUGAGGCGGAAAGGCGGAA	AACUAUU

Table 35: RSV (N) HH Target Sequence

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	GGCAAAU A CAAAGAU	217	GGUADGU U AUNDGOG
21	GAUGGCU C UUAGCAA	218	GUADGUU A UAUGCGA
23	UGGCUU U AGCAAAG	220	AUGGUU A UGCGAUG
24	GGCUU A GCAAAGU	229	GCGADGU C UAGGUUA
32	GCAAAGU C AAGUUGA	231	GAUGCU A GGUUAGG
37	GUCAAGU U GAUAGAU	235	UCUAGGU U AGGAAGA
45	GAUAGAU A CACUCAA	236	CUAGGUU A GGAAGAG
50	AUACAU C AACAAAG	254	ACACCAU A AAAADAC
60	CAAAGAU C AACUUCU	260	UAAAAU A CUCAGAG
65	AUCAAU U CGUCAU	263	AAADACU C AGAGAGG
66	UCAACU C UGUCADC	277	GCGGUU A UCAUGUA
70	CUUCGU C AUCCAGC	279	GGGUU C AUGUAAA
73	CGUCAU C CAGCAAA	284	AUCAU A AAGCAA
82	AGCAAU A CACCAUC	299	AUGGAGU A GAUGUAA
89	ACACCAU C CAACGA	305	UAGAGU A ACAACAC
108	AGGAGU A GUADUGA	315	AACCAU C GUCAAGA
111	AGAUAGU A UUGADAC	318	ACADGU C AAGACAU
113	AUAGAU U GAUACUC	326	AAGCAU U AAUGGAA
117	UADUGAU A CUCUAAA	327	AGCAU A AUGGAAA
120	UGADACU C CUADUUA	346	AUGAAU U UGAAGUG
123	UAUCU C AUUADGA	347	UGAAAU U GAAGUGU
126	UCCUAAU U AUGADGU	355	GAAGUGU U AACAUUG
127	CCUAAU A UGAUGUG	356	AAGUGU A ACAUGUG
146	AACCAU C AAUAAGU	361	UUAACAU U GGCAGC
150	CAUCAU A AGUUDUG	370	GCAAGCU U AACAAU
154	AAUAAGU U AUGUGGC	371	CAAGCU A ACAACUG
155	AUAAGU A UGUGGCA	383	CGAAU U CAADCA
166	GGCAUGU U AUUADC	384	UGAAAU C AAADCAA
167	GCAUGU A UUAADCA	389	UUCAAU C AACAUUG
169	AUGUUAU U AAUCACA	395	UCAACAU U GAGUAG
170	UGUUAU A AUCACAG	401	UUGAGU A GAUCUA
173	UAUUAU C ACAGAG	406	AUAGAU C UGAAAA
186	AGAUGCU A AUCAUAA	408	AGAAU C GAAADC
189	UGCUAU C AUAAAU	415	AGAAAU C CUACAAA
192	UAUACAU A AAUOCAC	418	AAAUCCU A CAAAAA
196	CAUAAU U CACUGGG	431	AAAUCCU A AAAGAAA
197	AUAAAU C ACUGGGU	449	GAGAGU A GUUCAG
205	ACUGGGU U AAUAGGU	453	GGUAGCU C CAGAAUA
206	CUGGGU A ADAGGUA	460	CCAGAU A CAGGCAU
209	GGUUAU A GGUAGU	472	CAUACU C UCCUGAU
213	AAUAGGU A UGUUUA	474	UGACU C CUGADUG

480	UCCUGAU U GUGGGAU	696	UUUUGGU A UAGCACA
491	GGADGAU A AUAUUAU	698	UUGGUU A GCACAAU
494	UGADAAU A UUAUGUA	706	GCACAAU C UUCUACC
496	AUAUAU U AUGUAUA	708	ACUADCU U CUUACCAG
497	UUAUAU A UGUADAG	709	CAUUCU C UACCAUA
501	AUAUAGU A UAGCAGC	711	AUCUUCU A CCAAGAG
503	UADGUU A GCAGCAU	726	UGGCAGU A GAGUUGA
511	GCAGCAU U AGUAUA	731	GUAGAGU U GAAGGGA
512	CAGCAU A GUAADAA	740	AAGGGAU U UUGGCAG
515	CAUAGU A AUAACUA	741	AGGGAU U UUGCAGG
518	UAGUAU A ACUAUAU	742	GGCAU U UGCAGGA
522	AUAUAU A AAUUAUC	743	GGAUU U GCAGGAU
526	ACUAUAU U AGCAGCA	751	GCAGGAU U GUUUAUG
527	CUAAAU A GCAGCAG	754	GGAUUGU U UADGAU
544	GACAGU C UGGUUCU	755	GADUGU U AUGAUG
549	AUCUGU C UACAGC	756	AUGGUU A UGAUUC
551	CUUGUCU U ACAGGCG	766	AUUGCU A UGGUGCA
552	UGGUCU A CAGGCGU	787	GUGAUGU U ACGGUGG
563	CGUGAU U AGGAGAG	788	UGAUGU A CGGUGG
564	CGUGAU A GGAGAGC	800	GGGAGU C UAGCAA
573	GAGAGU A AUAUAGU	802	GGAGU C AGCAAAA
576	AGCUAU A AUGUUCU	803	GAGUCU A GCAAAAU
581	AUAUAGU C CUA AAAA	811	GCAAAU C AGUAAA
584	AUGUCCU A AAAAAG	815	AUUCAGU U AAAAUA
603	GAAGCU U ACAAGG	816	AUCAGU A AAAAUA
604	AAAGCU A CAAGGC	822	UAAAAU A UUAUGU
613	AAAGCU U ACUACCC	824	AAAAUA U AUGUAG
614	AAGGCU A CUACCCA	825	AAUAU A UGUUAGG
617	GCUAU A CCAAGG	829	AUAUAGU U AGGACAU
629	AGGACU A GCAACA	830	UUAUGU A GGCAGG
640	AACAGU U CUUAGAA	840	ACADGU A GUGUGCA
641	ACAGCU C UADGAAG	866	AACAGU U GUUGAGG
643	AGCUUCU A UGAAGUG	869	AAGUGU U GAGGUU
652	GAGUGU U UGAAAA	875	UUGAGGU U UADGAU
653	AAGUGU U GAAAAAC	876	UGAGGU U AUGAUA
663	AAAAAU C CCAACU	877	GAGGUU A UGAUAU
670	CCCAU U UADGAU	883	UADGAU A UGCCAA
671	CCCAU U ADAGAG	895	CAAAAAU U GGGUGGU
672	CCCAU A UAGAUU	913	GCAGAU U CUUCCAU
674	ACUUUAU A GAUGUU	914	CAGAU C UACCAUA
680	UAGAUU U UUGUUC	916	GGAUU A CCAUAUA
681	AGAUGU U UUGUCCA	921	CUUCCAU A UAUUGA
682	GAUGUU U UGUUCAU	923	ACCAUA A UGAACA
683	AUGUUU U GUUUAU	925	CAUAUA U GAACAAC
686	UUUUGU C CAUUUG	943	AAAGCAU C AUUAUA
687	UUUGU C AUUUUG	946	GCAUCAU U AUUAU
690	UGUUCU U UUGUAU	947	CAUCAU A UUAUCU
691	GUUCAU U UGGUAUA	949	UCAUAU U AUUUUG
692	UUCAUU U GGUADAG	950	CAUAUA A UCUUGA

952	UUUUUU C UUUUUU
954	UUUUUU U UUUUUU
955	UUUUUU C UUUUUU
960	UUUUUU C UUUUUU
964	UUUUUU U UUUUUU
965	UUUUUU U UUUUUU
966	UUUUUU C UUUUUU
969	UUUUUU C UUUUUU
973	UUUUUU U UUUUUU
974	UUUUUU C UUUUUU
976	UUUUUU C UUUUUU
983	UUUUUU A UUUUUU
986	UUUUUU A UUUUUU
988	UUUUUU U UUUUUU
989	UUUUUU A UUUUUU
1007	UUUUUU A UUUUUU
1013	UUUUUU A UUUUUU
1024	UUUUUU A UUUUUU
1032	UUUUUU A UUUUUU
1044	UUUUUU C UUUUUU
1050	UUUUUU C UUUUUU
1052	UUUUUU A UUUUUU
1054	UUUUUU A UUUUUU
1072	UUUUUU A UUUUUU
1085	UUUUUU C UUUUUU
1103	UUUUUU U UUUUUU
1104	UUUUUU A UUUUUU
1108	UUUUUU A UUUUUU
1115	UUUUUU A UUUUUU
1118	UUUUUU A UUUUUU
1123	UUUUUU U UUUUUU
1139	UUUUUU A UUUUUU
1146	UUUUUU A UUUUUU
1148	UUUUUU C UUUUUU
1155	UUUUUU C UUUUUU
1160	UUUUUU U UUUUUU
1161	UUUUUU A UUUUUU
1164	UUUUUU C UUUUUU
1173	UUUUUU A UUUUUU
1181	UUUUUU A UUUUUU
1187	UUUUUU U UUUUUU
1188	UUUUUU U UUUUUU
1193	UUUUUU U UUUUUU
1194	UUUUUU A UUUUUU

Table 36: RSV (N) HH Ribozyme Sequence

nt. Position	HH Ribozyme Sequence
9	AUCUUG CUGAUGAGGCGAAAGGCGGAA AUUUGCC
21	UUGCUAA CUGAUGAGGCGAAAGGCGGAA AGCCAUU
23	CUUUGCU CUGAUGAGGCGAAAGGCGGAA AGAGCCA
24	ACUUGUC CUGAUGAGGCGAAAGGCGGAA AAGAGCC
32	UCUACUU CUGAUGAGGCGAAAGGCGGAA ACUUGCC
37	AUCAUUC CUGAUGAGGCGAAAGGCGGAA ACUUGAC
45	UUGAGUG CUGAUGAGGCGAAAGGCGGAA AUCAUUC
50	CUUUGUU CUGAUGAGGCGAAAGGCGGAA AGUGUAU
60	AGAAGUU CUGAUGAGGCGAAAGGCGGAA AUUUUGG
65	AUGACAG CUGAUGAGGCGAAAGGCGGAA AGUUGAU
66	GADGACA CUGAUGAGGCGAAAGGCGGAA AAGUUGA
70	GCGGGAU CUGAUGAGGCGAAAGGCGGAA ACAGAAG
73	UUUGCUG CUGAUGAGGCGAAAGGCGGAA AUGACAG
82	GADGGUG CUGAUGAGGCGAAAGGCGGAA AUUUGCU
89	UCCGUUG CUGAUGAGGCGAAAGGCGGAA AUGGUGU
108	UCAUAUC CUGAUGAGGCGAAAGGCGGAA AUUUCCU
111	GUADCAA CUGAUGAGGCGAAAGGCGGAA ACUAUCU
113	GAGUAUC CUGAUGAGGCGAAAGGCGGAA AUACUAU
117	UUGAGAG CUGAUGAGGCGAAAGGCGGAA AUCAADA
120	UAADUAG CUGAUGAGGCGAAAGGCGGAA AGUAACA
123	UCAUAU CUGAUGAGGCGAAAGGCGGAA AGGAGUA
126	ACAUCAU CUGAUGAGGCGAAAGGCGGAA AUUAGGA
127	CACAUCA CUGAUGAGGCGAAAGGCGGAA AAUUGGG
146	ACUUAUU CUGAUGAGGCGAAAGGCGGAA AUGUGUU
150	CAUAACU CUGAUGAGGCGAAAGGCGGAA AUUGAUG
154	GCCACAU CUGAUGAGGCGAAAGGCGGAA ACUUAUU
155	UGCCACA CUGAUGAGGCGAAAGGCGGAA AACUUAU
166	GADUAAU CUGAUGAGGCGAAAGGCGGAA ACAUGCC
167	UGAUUAA CUGAUGAGGCGAAAGGCGGAA AACAUCC
169	UGUGAUU CUGAUGAGGCGAAAGGCGGAA AUUACAU
170	CUGUGAU CUGAUGAGGCGAAAGGCGGAA AAUAACA
173	CUUCUGU CUGAUGAGGCGAAAGGCGGAA AUUAADA
186	UUUUGAU CUGAUGAGGCGAAAGGCGGAA AGCAUUC
189	AAUUUAU CUGAUGAGGCGAAAGGCGGAA AUUAGCA
192	GUGAAUU CUGAUGAGGCGAAAGGCGGAA AUGAUUA
196	COCAGUG CUGAUGAGGCGAAAGGCGGAA AUUUADG
197	AOCAGU CUGAUGAGGCGAAAGGCGGAA AAUUUAU
205	ACCUAAU CUGAUGAGGCGAAAGGCGGAA AOCAGU
206	UACCUAU CUGAUGAGGCGAAAGGCGGAA AACCCAG
209	ACAUAAC CUGAUGAGGCGAAAGGCGGAA AUUAACC
213	UAUAACA CUGAUGAGGCGAAAGGCGGAA ACCUAUU

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217	CGCAU	CUGAUGAGGCGAAAGGCGAA	ACAUAAC
218	UGCAUA	CUGAUGAGGCGAAAGGCGAA	AACAUAAC
220	CADCGCA	CUGAUGAGGCGAAAGGCGAA	AUAACAUA
229	UAACCUA	CUGAUGAGGCGAAAGGCGAA	ACADCGC
231	CCUAACC	CUGAUGAGGCGAAAGGCGAA	AGACAUC
235	UCUUCU	CUGAUGAGGCGAAAGGCGAA	ACCUAGA
236	CUCUCC	CUGAUGAGGCGAAAGGCGAA	AACUAG
254	GUUUUU	CUGAUGAGGCGAAAGGCGAA	AUGGUGU
260	CUCGAG	CUGAUGAGGCGAAAGGCGAA	AUUUUUA
263	CADCCU	CUGAUGAGGCGAAAGGCGAA	AGUAUUU
277	UACADGA	CUGAUGAGGCGAAAGGCGAA	AUCCCGC
279	UUACAUA	CUGAUGAGGCGAAAGGCGAA	AUAUCC
284	UUGCUUU	CUGAUGAGGCGAAAGGCGAA	ACADGAU
299	UACAUUC	CUGAUGAGGCGAAAGGCGAA	ACUCCAU
305	GUGUUGU	CUGAUGAGGCGAAAGGCGAA	ACAUCUA
315	UCUUGAC	CUGAUGAGGCGAAAGGCGAA	AUGUGUU
318	AUGCCUU	CUGAUGAGGCGAAAGGCGAA	ACGAUGU
326	UUCCAUU	CUGAUGAGGCGAAAGGCGAA	AUGUCUU
327	UUCCAU	CUGAUGAGGCGAAAGGCGAA	AUGUCUU
346	CACUCCA	CUGAUGAGGCGAAAGGCGAA	AUUUCAU
347	ACACUUC	CUGAUGAGGCGAAAGGCGAA	AUUUCA
355	CAUGUUU	CUGAUGAGGCGAAAGGCGAA	ACACUUC
356	CCAUGUU	CUGAUGAGGCGAAAGGCGAA	AACACUU
361	GUUGCC	CUGAUGAGGCGAAAGGCGAA	AUGUUAA
370	AGUUGUU	CUGAUGAGGCGAAAGGCGAA	AGUUGC
371	CAGUUGU	CUGAUGAGGCGAAAGGCGAA	AGCUUG
383	UGAUUG	CUGAUGAGGCGAAAGGCGAA	AUUUCAG
384	UGAUUU	CUGAUGAGGCGAAAGGCGAA	AUUUCA
389	CAUGUU	CUGAUGAGGCGAAAGGCGAA	AUUUGAA
395	CUAUCU	CUGAUGAGGCGAAAGGCGAA	AUGUUGA
401	UAGAUUC	CUGAUGAGGCGAAAGGCGAA	AUCUCA
406	UUUUCUA	CUGAUGAGGCGAAAGGCGAA	AUUUCUA
408	GAUUUC	CUGAUGAGGCGAAAGGCGAA	AGAUUCU
415	UUUGUAG	CUGAUGAGGCGAAAGGCGAA	AUUUCU
418	UUUUUG	CUGAUGAGGCGAAAGGCGAA	AGGAUUU
431	UUUCUUU	CUGAUGAGGCGAAAGGCGAA	AGCAUUU
449	CUUGAGC	CUGAUGAGGCGAAAGGCGAA	ACUUCUC
453	UAUUCUG	CUGAUGAGGCGAAAGGCGAA	AGCUAAC
460	AUGCCUG	CUGAUGAGGCGAAAGGCGAA	AUUUCUG
472	AUCAGGA	CUGAUGAGGCGAAAGGCGAA	AGUCAUG
474	CAUCAG	CUGAUGAGGCGAAAGGCGAA	AGAGUCA
480	AUCCAC	CUGAUGAGGCGAAAGGCGAA	AUCAGGA
491	AUAUAUA	CUGAUGAGGCGAAAGGCGAA	AUCAUCC
494	UACAUA	CUGAUGAGGCGAAAGGCGAA	AUUUAUA
496	UAUAUAU	CUGAUGAGGCGAAAGGCGAA	AUAUAUA
497	CUAUACA	CUGAUGAGGCGAAAGGCGAA	AUAUAUA
501	GUUGCUA	CUGAUGAGGCGAAAGGCGAA	ACAUAUA
503	AUGCUGC	CUGAUGAGGCGAAAGGCGAA	AUAUAUA
511	UAUUAUA	CUGAUGAGGCGAAAGGCGAA	AUGCUGC

512	UUADUAC	CUGAUGAGGCGGAAAGGCGGAA	AADGCUG
515	UAGGUAU	CUGADGAGGCGGAAAGGCGGAA	ACTAADG
518	AUUUAGU	CUGADGAGGCGGAAAGGCGGAA	AUUACUA
522	GUUAAU	CUGADGAGGCGGAAAGGCGGAA	AGUUU
526	UGCUGCU	CUGADGAGGCGGAAAGGCGGAA	AUUUAGU
527	CUGCUGC	CUGADGAGGCGGAAAGGCGGAA	AUUUAG
544	AAGACCA	CUGADGAGGCGGAAAGGCGGAA	AUCUGUC
549	GUUGUA	CUGADGAGGCGGAAAGGCGGAA	ACCAU
551	CGGUGU	CUGADGAGGCGGAAAGGCGGAA	AGACAG
552	AGGUGU	CUGADGAGGCGGAAAGGCGGAA	AAGACCA
563	CUUCCU	CUGADGAGGCGGAAAGGCGGAA	AUCACG
564	GUUCCU	CUGADGAGGCGGAAAGGCGGAA	AUCACG
573	ACAUU	CUGADGAGGCGGAAAGGCGGAA	AGUUCU
576	AGGACU	CUGADGAGGCGGAAAGGCGGAA	AUUAGCU
581	UUUUUAG	CUGADGAGGCGGAAAGGCGGAA	ACAUU
584	CAUUUU	CUGADGAGGCGGAAAGGCGGAA	AGGACU
603	CUUUUU	CUGADGAGGCGGAAAGGCGGAA	AGGUUC
604	GUUUUG	CUGADGAGGCGGAAAGGCGGAA	AAGUUU
613	GGGUAGU	CUGADGAGGCGGAAAGGCGGAA	AGUUUU
614	UGGUUAG	CUGADGAGGCGGAAAGGCGGAA	AAGUUU
617	CUUUGG	CUGADGAGGCGGAAAGGCGGAA	AGUAGC
629	UGUUGG	CUGADGAGGCGGAAAGGCGGAA	AGUUCU
640	UUCAUAG	CUGADGAGGCGGAAAGGCGGAA	AGUUGU
641	CUUCAU	CUGADGAGGCGGAAAGGCGGAA	AAGUGU
643	CACUUA	CUGADGAGGCGGAAAGGCGGAA	AGUAGU
652	UUUUUA	CUGADGAGGCGGAAAGGCGGAA	ACAUUC
653	GUUUUC	CUGADGAGGCGGAAAGGCGGAA	AACAUU
663	AAGUGG	CUGADGAGGCGGAAAGGCGGAA	AGUUUU
670	AUCUUA	CUGADGAGGCGGAAAGGCGGAA	AGUGGG
671	CAUCU	CUGADGAGGCGGAAAGGCGGAA	AAGUGG
672	ACAUUA	CUGADGAGGCGGAAAGGCGGAA	AAAGUG
674	AAACAU	CUGADGAGGCGGAAAGGCGGAA	AUAAGU
680	GAACAA	CUGADGAGGCGGAAAGGCGGAA	ACAUUA
681	UGAACAA	CUGADGAGGCGGAAAGGCGGAA	AACAUU
682	AUGAAC	CUGADGAGGCGGAAAGGCGGAA	AAACAU
683	AUGAAC	CUGADGAGGCGGAAAGGCGGAA	AAACAU
686	CAAAAU	CUGADGAGGCGGAAAGGCGGAA	ACAAAA
687	CCAAAU	CUGADGAGGCGGAAAGGCGGAA	AACAAA
690	AUAACAA	CUGADGAGGCGGAAAGGCGGAA	AUGAAC
691	UAUACCA	CUGADGAGGCGGAAAGGCGGAA	AUGAAC
692	CUAUAC	CUGADGAGGCGGAAAGGCGGAA	AAUGAA
696	UGUGUA	CUGADGAGGCGGAAAGGCGGAA	ACCAAA
698	AUGUGC	CUGADGAGGCGGAAAGGCGGAA	AUAACAA
706	GGUAGAA	CUGADGAGGCGGAAAGGCGGAA	AUGUGC
708	CGGUAG	CUGADGAGGCGGAAAGGCGGAA	AGUUGU
709	UCUGUA	CUGADGAGGCGGAAAGGCGGAA	AAGUUG
711	CCUGUG	CUGADGAGGCGGAAAGGCGGAA	AGUAGU
726	UCAACU	CUGADGAGGCGGAAAGGCGGAA	ACUGUA
731	UCCUUC	CUGADGAGGCGGAAAGGCGGAA	ACUUC

740	CUGCAA	CUGAUGAGGCGGAAAGGCGGAA	AUCCCUU
741	CCUGCA	CUGAUGAGGCGGAAAGGCGGAA	AAUCCCU
742	UCCUGCA	CUGAUGAGGCGGAAAGGCGGAA	AAAUCCC
743	AUCCUGC	CUGAUGAGGCGGAAAGGCGGAA	AAAADCC
751	CADAAAC	CUGAUGAGGCGGAAAGGCGGAA	AUCCUGC
754	AUUCAU	CUGAUGAGGCGGAAAGGCGGAA	ACAADCC
755	CAUUCU	CUGAUGAGGCGGAAAGGCGGAA	AACAADC
756	GCAUUA	CUGAUGAGGCGGAAAGGCGGAA	AAACAAD
766	UGCAUUA	CUGAUGAGGCGGAAAGGCGGAA	AGGCAAD
787	CCACUUA	CUGAUGAGGCGGAAAGGCGGAA	ACAUCAC
788	CCACUUA	CUGAUGAGGCGGAAAGGCGGAA	AACAUCA
800	UUGCUAA	CUGAUGAGGCGGAAAGGCGGAA	ACUCCUC
802	UUUUGCU	CUGAUGAGGCGGAAAGGCGGAA	AGAUCUC
803	AUUUUGC	CUGAUGAGGCGGAAAGGCGGAA	AAGACUC
811	UUUAACU	CUGAUGAGGCGGAAAGGCGGAA	AUUUUGC
815	UAUUUUU	CUGAUGAGGCGGAAAGGCGGAA	ACUGAUC
816	AUAUUUU	CUGAUGAGGCGGAAAGGCGGAA	AACUGAU
822	AACAUA	CUGAUGAGGCGGAAAGGCGGAA	AUUUUUA
824	CUAACA	CUGAUGAGGCGGAAAGGCGGAA	AUAUUUU
825	CCUAACA	CUGAUGAGGCGGAAAGGCGGAA	AUAUUUU
829	AUGUCCU	CUGAUGAGGCGGAAAGGCGGAA	ACAUAUU
830	CAUGUCC	CUGAUGAGGCGGAAAGGCGGAA	AACAUAU
840	UGCACAC	CUGAUGAGGCGGAAAGGCGGAA	AGCAUCU
866	CCUCAAC	CUGAUGAGGCGGAAAGGCGGAA	ACUUGUU
869	AAACUCC	CUGAUGAGGCGGAAAGGCGGAA	ACAACUU
875	AUUCUA	CUGAUGAGGCGGAAAGGCGGAA	ACUUCUA
876	UAUUCU	CUGAUGAGGCGGAAAGGCGGAA	AACUUA
877	AUAUUA	CUGAUGAGGCGGAAAGGCGGAA	AAACUUC
883	UUGGCUA	CUGAUGAGGCGGAAAGGCGGAA	AUUCUAU
895	AACACCC	CUGAUGAGGCGGAAAGGCGGAA	AUUUUUG
913	AUGGUAG	CUGAUGAGGCGGAAAGGCGGAA	AUCCUGC
914	UAUGGUA	CUGAUGAGGCGGAAAGGCGGAA	AAUCCUG
916	UAUAUGG	CUGAUGAGGCGGAAAGGCGGAA	AGAADC
921	UUCUAUA	CUGAUGAGGCGGAAAGGCGGAA	AUGGUAG
923	UGUUCUA	CUGAUGAGGCGGAAAGGCGGAA	AUAUGGU
925	GUUGUUC	CUGAUGAGGCGGAAAGGCGGAA	AUAUAUG
943	UAADAAU	CUGAUGAGGCGGAAAGGCGGAA	AUGCUUU
946	AGADAAU	CUGAUGAGGCGGAAAGGCGGAA	AUGADGC
947	AAGADAA	CUGAUGAGGCGGAAAGGCGGAA	AAUGAUG
949	CAAAGAU	CUGAUGAGGCGGAAAGGCGGAA	AUAADGA
950	UCAAGA	CUGAUGAGGCGGAAAGGCGGAA	AUAADUG
952	AGUCAAA	CUGAUGAGGCGGAAAGGCGGAA	AUAADAA
954	UGAGUCA	CUGAUGAGGCGGAAAGGCGGAA	AGAUAAU
955	UUGAGUC	CUGAUGAGGCGGAAAGGCGGAA	AAGADAA
960	GGAAAUU	CUGAUGAGGCGGAAAGGCGGAA	AGUCAAA
964	GUGAGGA	CUGAUGAGGCGGAAAGGCGGAA	AUUGAGU
965	AGUGAGG	CUGAUGAGGCGGAAAGGCGGAA	AAUUGAG
966	AAGUGAG	CUGAUGAGGCGGAAAGGCGGAA	AAAUUGA
969	GAGAAGU	CUGAUGAGGCGGAAAGGCGGAA	AGGAAAU

973	ACUGGAG	CUGAUGAGGCGGAAAGGCGGAA	AGUGAGG
974	CACUGGA	CUGAUGAGGCGGAAAGGCGGAA	AAGUGAG
976	UACACUG	CUGAUGAGGCGGAAAGGCGGAA	AGAAGUG
983	CUAUAC	CUGAUGAGGCGGAAAGGCGGAA	ACACUGG
986	UGOCUAA	CUGAUGAGGCGGAAAGGCGGAA	ACUACAC
988	AUUGOCU	CUGAUGAGGCGGAAAGGCGGAA	AUAUAC
989	CAUUGOC	CUGAUGAGGCGGAAAGGCGGAA	AUAUAC
1007	UUAUGOC	CUGAUGAGGCGGAAAGGCGGAA	AGGOCAG
1013	CUCCCAU	CUGAUGAGGCGGAAAGGCGGAA	AUGCCUA
1024	ACUUCUG	CUGAUGAGGCGGAAAGGCGGAA	ACUCGCC
1032	CUCCGUG	CUGAUGAGGCGGAAAGGCGGAA	ACCCUGG
1044	AGAUCUU	CUGAUGAGGCGGAAAGGCGGAA	AUUCGUC
1050	UCAUADA	CUGAUGAGGCGGAAAGGCGGAA	AUCUUGA
1052	CADCAUA	CUGAUGAGGCGGAAAGGCGGAA	AGAUCUU
1054	UGCAUCA	CUGAUGAGGCGGAAAGGCGGAA	AUAUAGC
1072	UUCAGCA	CUGAUGAGGCGGAAAGGCGGAA	AUGCCUU
1085	UUUCUUU	CUGAUGAGGCGGAAAGGCGGAA	AGUUGUU
1103	UGUAGUU	CUGAUGAGGCGGAAAGGCGGAA	AUCACAC
1104	CUGUAGU	CUGAUGAGGCGGAAAGGCGGAA	AUUCACA
1108	UACACUG	CUGAUGAGGCGGAAAGGCGGAA	AGUUAUU
1115	AGUCUAG	CUGAUGAGGCGGAAAGGCGGAA	ACACUGU
1118	UCAAGUC	CUGAUGAGGCGGAAAGGCGGAA	AGUACAC
1123	UGCUGUC	CUGAUGAGGCGGAAAGGCGGAA	AGGCCAG
1139	UAGCCUC	CUGAUGAGGCGGAAAGGCGGAA	AGUUCUU
1146	UGUUUCA	CUGAUGAGGCGGAAAGGCGGAA	AGCCUCU
1148	GAUGUUU	CUGAUGAGGCGGAAAGGCGGAA	AUAAGCU
1155	UUAAGCU	CUGAUGAGGCGGAAAGGCGGAA	AUGUUUG
1160	UUGGAUU	CUGAUGAGGCGGAAAGGCGGAA	AGCUGAU
1161	UUUGGAU	CUGAUGAGGCGGAAAGGCGGAA	AAGCUGA
1164	UCUUUGG	CUGAUGAGGCGGAAAGGCGGAA	AUAAGC
1173	ACAUCAU	CUGAUGAGGCGGAAAGGCGGAA	AUCUUUU
1181	AAAGCUC	CUGAUGAGGCGGAAAGGCGGAA	ACAUCAU
1187	UAAUCA	CUGAUGAGGCGGAAAGGCGGAA	AGCCUCA
1188	UUAACUC	CUGAUGAGGCGGAAAGGCGGAA	AAGCUCU
1193	UUUUAUU	CUGAUGAGGCGGAAAGGCGGAA	ACUCAAA
1194	UUUUUAU	CUGAUGAGGCGGAAAGGCGGAA	AACUCAA

Table 87: RSV (1B) HP Ribozyme/Substrate Sequence

nt. Position	HP Ribozyme Sequence	Substrate
70	CUGUUAUC AGAA GUCUUU ACCAAGAGAAACACACGUGUGUGUACAUUACCUUGUA	AAAGACU GAU GAUCACAG
91	CAGUUAAC AGAA GUCUUA ACCAAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UAGAACU GUU GUCACUUG
472	CAGGCUCC AGAA GAGCUA ACCAAGAGAAACACACGUGUGUGUACAUUACCUUGUA	UAGUCCA GAU GAGGCUUG

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Table 38: RSV (N) HP Ribozyme/Substrate Sequence

nt. Position	Hairpin Ribozyme Sequence	Substrate
476	AUCCACCA AGAA GGAAGG ACCAGAGAAACACACGUGUGGUAACAUAUACCUAGUA	CUCUCCU GAU UGUUGGAU
540	AAGAACCA AGAA GUCCCC ACCAGAGAAACACACGUGUGGUAACAUAUACCUAGUA	GGGUAUA GAU CUGGUCUU
554	CUAAUCAC AGAA GUAAAG ACCAGAGAAACACACGUGUGGUAACAUAUACCUAGUA	UUUUUA GCC GUAAUUAG
636	UUCAUAGA AGAA GUUGGC ACCAGAGAAACACACGUGUGGUAACAUAUACCUAGUA	GGCAACA GCU UCUAAUUA
998	CCUAGGCC AGAA GCAUUG ACCAGAGAAACACACGUGUGGUAACAUAUACCUAGUA	CAUUGCU GCU GGGCUAGG
1156	UUGCAUUA AGAA GAUUGU ACCAGAGAAACACACGUGUGGUAACAUAUACCUAGUA	AACAUA GCU UAAUCCAA

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Table 39: Large-Scale Synthesis

Sequence	Activator [Added/Final] (min)	Amidite [Added/Final] (min)	Time*	% Full Length Product
A ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	85
A ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	89
(GGU) ₃ GGT	T [0.50/0.33]	[0.1/0.02]	15 m	78
(GGU) ₃ GGT	S [0.25/0.17]	[0.1/0.02]	15 m	81
C ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	90
C ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	97
U ₉ T	T [0.50/0.33]	[0.1/0.02]	15 m	80
U ₉ T	S [0.25/0.17]	[0.1/0.02]	15 m	85
A (36-mer)	T [0.50/0.33]	[0.1/0.02]	15/15m	21
A (36-mer)	S [0.25/0.17]	[0.1/0.02]	15/15 m	25
A (36-mer)	S [0.50/0.24]	[0.1/0.03]	15/15 m	25
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	15/15 m	38
A (36-mer)	S [0.50/0.18]	[0.1/0.05]	10/5 m	42

*Where two coupling times are indicated the first refers to RNA coupling and the second to 2'-O-methyl coupling. S = 5-S-Ethyltetrazole, T = tetrazole activator. A is 5'-ucu ccA UCU GAU GAG GCC GAA AGG CCG AAA AUC ccu -3' where lowercase represents 2'-O-methylnucleotides.

Table 40: Base Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
iBu(GGU) ₄	NH ₄ OH/EtOH	16 h	55	62.5
	MA	10 m	65	62.7
	AMA	10 m	65	74.8
	MA	10 m	55	75.0
	AMA	10 m	55	77.2
iPrP(GGU) ₄	NH ₄ OH/EtOH	4 h	65	44.8
	MA	10 m	65	65.9
	AMA	10 m	65	59.8
	MA	10 m	55	61.3
	AMA	10 m	55	60.1
C ₉ U	NH ₄ OH/EtOH	4 h	65	75.2
	MA	10 m	65	79.1
	AMA	10 m	65	77.1
	MA	10 m	55	79.8
	AMA	10 m	55	75.5
A (36-mer)	NH ₄ OH/EtOH	4 h	65	22.7
	MA	10 m	65	28.9

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Table 41: 2'-O-Alkylsilyl Deprotection

Sequence	Deprotection Reagent	Time (min)	T °C	% Full Length Product
AgT	TBAF	24 h	20	84.5
	1.4 M HF	0.5 h	65	81.0
(GGU) ₄	TBAF	24 h	20	60.9
	1.4 M HF	0.5 h	65	67.8
C ₁₀	TBAF	24 h	20	86.2
	1.4 M HF	0.5 h	65	86.1
U ₁₀	TBAF	24 h	20	84.8
	1.4 M HF	0.5 h	65	84.5
B (36-mer)	TBAF	24 h	20	25.2
	1.4 M HF	1.5 h	65	30.6
A (36-mer)	TBAF	24 h	20	29.7
	1.4 M HF	1.5 h	65	30.4

B is 5'- UCU CCA UCU GAU GAG GCC GAA AGG CCG AAA AUC CCU

-3'.

Table 42: NMR Data for UC Dimers containing
Phosphorothioate Linkage

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3524	ribo	2 x 3 s	10.4	2 x 100 s	95.9
3525	ribo	2 x 3 s	10.4	2 x 75 s	92.6
3530	ribo	2 x 3 s	10.4	2 x 75 s	92.1
3526	ribo	1 x 5 s	08.6	1 x 300 s	100.0
3578	ribo	1 x 5 s	08.6	1 x 250 s	100.0
3529	ribo	1 x 5 s	08.6	1 x 150 s	73.7

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Table 43: *NMR Data for 15-mer RNA containing
Phosphorothioate Linkages*

Synthesis #	Type	Delivery	Eq.	Wait	ASE (%)
3581	ribo	1 x 5 s	08.6	1 x 250 s	99.6
3663	ribo	2 x 4 s	13.8	2 x 300 s	100.0
3582	2'-O-Me	1 x 5 s	08.6	1 x 250 s	99.7
3668	2'-O-Me	2 x 4 s	13.8	2 x 300 s	99.8
3682	2'-O-Me	1 x 5 s	08.6	1 x 300 s	99.8

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Table 44. Kinetics of Self-Processing *In Vitro*

Self-Processing Constructs	k (min ⁻¹)*
HH	1.16 ± 0.08
HDV	0.56 ± 0.15
HP(GC)	0.36 ± 0.06
HP(GU)	0.054 ± 0.003

* k represents the unimolecular rate constant for ribozyme self-cleavage determined from a non-linear, least-squares fit (KaleidaGraph, Synergy Software, Reading, PA) to the equation:

$$(\text{Fraction Uncleaved Transcript}) = \frac{1}{kt} (1 - e^{-kt})$$

The equation describes the extent of ribozyme processing in the presense of ongoing transcription (Long & Uhlenbeck, 1994 Proc. Natl. Acad. Sci. USA 91, 6977) as a function of time (t) and the unimolecular rate constant for cleavage (k). Each value of k represents the average (± range) of values determined from two experiments.

Table 45

Entry	Modification	$t_{1/2}$ (m) Activity (t_A)	$t_{1/2}$ (m) Stability (t_S)	$\beta = t_S/t_A$ $\times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-O-Me-U	4	260	650
3	U4 = 2'-CH ₂ -U	6.5	120	180
4	U7 = 2'-CH ₂ -U	8	280	350
5	U4 & U7 = 2'-CH ₂ -U	9.5	120	130
6	U4 = 2'-CF ₂ -U	5	320	640
7	U7 = 2'-CF ₂ -U	4	220	550
8	U4 & U7 = 2'-CF ₂ -U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH ₂ -U	10	500	500
19	U7 = 2'-NH ₂ -U	5	500	1000
20	U4 & U7 = 2'-NH ₂ -U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

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CLAIMSWhat is claimed is:

1. An enzymatic nucleic acid molecule which cleaves ICAM-1 mRNA, IL-5 mRNA, *rel A* mRNA, TNF- α mRNA sites shown in Table 23, 25, 27, or 28, CML associated mRNA selected from those identified as SEQ. ID NOS 1-25, or RSV mRNA or RSV genomic RNA in a region selected from the group consisting of 1C, 1B and N.
2. The enzymatic nucleic acid molecule of claim 1, the binding arms of which contain sequences complementary to any one of the sequences defined in any of those in Tables 2, 3, 6-9, 11, 13, 15-23, 27, 28, 31, 33, 34, 36, and 37.
3. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said nucleic acid molecule is in a hammerhead motif.
4. The enzymatic nucleic acid molecule of claim 1 or 2, wherein said RNA molecule is in a hairpin, hepatitis delta virus, group 1 intron, *Neurospora* VS RNA or RNaseP RNA motif.
5. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 12 and 100 bases complementary to said mRNA or genomic RNA.
6. The enzymatic nucleic acid molecule of claim 5 comprising between 14 and 24 bases complementary to said mRNA or genomic RNA.
7. The enzymatic nucleic acid molecule of claim 1 or 2, comprising between 5 and 23 bases complementary to said mRNA or genomic RNA.
8. The enzymatic nucleic acid molecule of claim 7 comprising between 10 and 18 bases complementary to said mRNA or genomic RNA.
9. An enzymatic nucleic acid molecule consisting essentially of a sequence selected from the group of those shown in Tables 4-8, 10, 12, 14-16, 19-22, 24, 26-28, 30, 32, 34 and 36-38.
10. A mammalian cell including an enzymatic nucleic acid molecule of claims 1 or 2.

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11. The cell of claim 10, wherein said cell is a human cell.
12. An expression vector including nucleic acid encoding an enzymatic nucleic acid molecule or multiple enzymatic molecules of claims 1 or 2 in a manner which allows expression of that enzymatic RNA molecule(s) within a mammalian cell.
13. A mammalian cell including an expression vector of claim 12.
14. The cell of claim 13, wherein said cell is a human cell.
15. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an enzymatic nucleic acid molecule of claim 1 or 2.
16. A method for treatment of a pathological condition related to the mRNA level of ICAM-1, IL-5, *rel A*, TNF- α , or RSV by administering to a patient an expression vector of claim 12.
17. The method of claims 15 or 16, wherein said patient is a human.
18. The method of claim 17 wherein said condition is selected from the group consisting of atherosclerosis, myocardial infraction, stroke, restenosis, heart diseases, cancer, rheumatoid arthritis, asthma, reperfusion injury, inflammatory or autoimmune disorders, transplant rejection, myocardial ischemia, stroke, psoriasis, Kawasaki disease, HIV and AIDS, and septic shock.
19. A nucleoside selected from the group consisting of 5'-C-alkylnucleoside, 2'-deoxy-2'-alkylnucleoside, nucleoside 5'-deoxy-5'-dihalo-methylphosphonate, nucleoside 5'-deoxy-5'-difluoro-methylphosphonate, nucleoside 3'-deoxy-3'-dihalo-methylphosphonate, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
20. A nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.

21. A nucleotide triphosphate comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
22. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in a talo configuration.
23. The 5'-C-alkylnucleoside of claim 19, wherein the sugar portion is in an allo configuration.
24. An oligonucleotide comprising a nucleotide selected from the group consisting of 5'-C-alkylnucleotide, 2'-deoxy-2'-alkylnucleotide, 5'-deoxy-5'-dihalo-methylnucleotide, 5'-deoxy-5'-difluoro-methylnucleotide, 3'-deoxy-3'-dihalo-methylnucleotide, and 5',3'-dideoxy-5',3'-bis(dihalo)-methylphosphonate.
25. An oligonucleotide comprising a moiety having the formula:
- wherein B is a nucleotide base or hydrogen; R1, R2 and R3 independently is selected from the group consisting of hydrogen, an alkyl group containing between 2 and 10 carbon atoms inclusive, an amine, an amino acid, and a peptide containing between 2 and 5 amino acids inclusive; and the zigzag lines are independently hydrogen or a bond.
26. An oligonucleotide comprising a 3'-amido or peptido group.
27. An oligonucleotide comprising a 5'-amido or peptido group.
28. The oligonucleotide of claim 24, 25, 26, or 27 having enzymatic activity.
29. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having an alkyl group at its 5'-position or 2'-position.

- 5 30. Method for conversion of a protected allo sugar to a protected talo sugar, comprising the step of contacting said protected allo sugar with triphenyl phosphine, diethylazodicarboxylate, p-nitrobenzoic acid under inversion causing conditions to provide said protected talo sugar.
- 10 31. Method for the synthesis of a nucleoside 5' or a 3'-dihalomethylphosphonate comprising the step of condensing a difluoromethylphosphonate-containing sugar with a pyrimidine or purine under conditions suitable for forming a nucleoside 5'- or 3'-difluoromethylphosphonate.
32. The oligonucleotide of claim 3, wherein the normal hammerhead U4 and/or U7 positions are substituted with 2'-NH-amino acid.
- 15 33. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at a delivered 0.1-1.0 M concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.
- 20 34. A method for the synthesis of RNA comprising the step of providing 5-S-alkyltetrazole at 0.15-0.35 M effective, or final, concentration for the activation of a RNA amidite during a coupling step for less than or equal to 10 minutes.
- 25 35. A method for the deprotection of RNA comprising the step of providing alkylamine (MA) or NH₄OH/alkylamine (AMA) at between 60°C - 70°C for 5 to 15 minutes to remove any exocyclic amino protecting groups from protected RNA; wherein said alkyl is selected from the group consisting of methyl, ethyl, propyl and butyl.
- 30 36. A method for the deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine-hydrogen fluoride (aHF•TEA) trimethylamine or diisopropylethylamine at between 60 °C-70 °C for 0.25-24 h.
37. A method for the purification of an RNA molecule by passing said enzymatic RNA molecule over an HPLC column, wherein said HPLC column is an anion exchange chromatography column.

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- 5 38. Method for one pot deprotection of RNA comprising, contacting a protected base with anhydrous methyl amine at between 60 °C-70 °C for at least 5 min, cooling the resulting mixture and contacting said mixture with TEA-3HF reagents under conditions which remove a protecting group of the 2'-hydroxyl position.
- 10 39. Method for synthesizing RNA containing a phosphorothioate linkage comprising the step of contacting 6-10 equivalents of 3H-1,2-benzodithiole-3-one 1,1-dioxide (Beaucage reagent) with the growing RNA chain for 5 seconds with a reaction time of at least 300 seconds.
40. Method of synthesizing RNA containing a phosphorothioate linkage comprising the step of achieving coupling with 5-S-ethyltetrazole or 5-S-methyltetrazole prior to sulfurization.
- 15 41. Method of claims 38, 39 or 40 wherein said RNA is enzymatically active.
42. Method for synthesizing 2'-deoxy-2'-amino-nucleoside phosphoramidite, comprising the step of protecting the 2'-amino group with a N-phtaloyl group.
43. The method of claim 42 wherein the said nucleoside lacks a base.
- 20 44. Method for synthesis of RNA comprising the step of: protecting the 2'-position of a nucleotide during said synthesis with a (trimethylsilyl)ethoxymethyl (SEM) group.
45. Method for covalently linking a SEM group to the 2'-position of a nucleotide, comprising the step of: contacting a nucleoside with an SEM-containing molecule under SEM bonding conditions.
- 25 46. The method of claim 45, wherein said conditions comprise dibutyltin oxide and tetrabutylammonium fluoride and SEM-Cl.
- 30 47. Method for removal of an SEM group from a nucleoside molecule or an oligonucleotide, comprising the step of: contacting said molecule or oligonucleotide with boron trifluoride etherate (BF₃·OEt₂) under SEM removing conditions.

48. The method of claim 57 wherein said (BF₃•OEt₂) is provided in acetonitrile.
49. One or more vectors comprising
- 5 a first nucleic acid sequence encoding a first ribozyme having intramolecular or intermolecular cleaving activity, said first ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif;
- 10 and a second nucleic acid sequence encoding a second ribozyme having intermolecular cleaving activity, said Second ribozyme being selected from the group consisting of a hammerhead, hairpin, hepatitis delta virus, *Neurospora* VS RNA, Group I, and RNaseP motif and said second nucleic acid being flanked by other nucleic acid sequences encoding RNA which is cleaved by said
- 15 first ribozyme to release said second ribozyme from RNA encoded by said vector;
- wherein said first and second nucleic acid sequences may be on the same or separate nucleic acid molecules, and said vector encodes mRNA or comprises RNA which lacks secondary structure which
- 20 reduces release of said second ribozyme by more than 20%.
50. Cell comprising the vector of claim 49.
51. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions
- 25 between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs.
52. The RNA molecule of claim 51, wherein said molecule is transcribed by a RNA polymerase III based promoter system.
53. The RNA molecule of claim 51, wherein said molecule is transcribed
- 30 by a type 2 pol III promoter system.
54. The RNA molecule of claim 51, wherein said molecule is a chimeric tRNA.

55. The RNA molecule of claim 53, said RNA having A and B boxes of a type 2 pol III promoter separated by between 0 and 300 bases.
56. The RNA molecule of claim 53, wherein said desired RNA molecule is at the 3' end of said B box.
- 5 57. The RNA molecule of claim 53, wherein said desired RNA molecule is in between the said A and the B box.
58. The RNA molecule of claim 53, wherein said desired RNA molecule includes said B box.
- 10 59. The RNA molecule of claim 51, wherein said desired RNA molecule is selected from the group consisting of antisense RNA, decoy RNA, therapeutic editing RNA, enzymatic RNA, agonist RNA and antagonist RNA.
60. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 12 bases of said 3' region.
- 15 61. The RNA molecule of claim 51, wherein said 5' terminus is able to base-pair with at least 15 bases of said 3' region.
62. DNA vector encoding the RNA molecule of claim 51
63. The vector of claim 62, wherein said vector is derived from an AAV or adeno virus.
- 20 64. RNA vector encoding the RNA molecule of claim 51.
65. The vector of claim 64, wherein said vector is derived from an alpha virus or retro virus.
66. The vector of claim 62 wherein the portions of the vector encoding said RNA function as a RNA pol III promoter.
- 25 67. Cell comprising the vector of claim 62.
68. Cell comprising the vector of claim 53.
69. Cell comprising the RNA of claim 51.

70. Method to provide a desired RNA molecule in a cell, comprising introducing said molecule into said cell a RNA comprising a desired RNA molecule, having a 5' terminus able to base pair with at least 8 bases of a 3' region of said RNA molecule.
- 5 71. The method of claim 70, wherein said introducing comprises providing a vector encoding said RNA molecule.
72. Hammerhead ribozyme having 2 or 3 base pairs in stem II with an interconnecting loop of 4 or more bases between said base pairs.
- 10 73. Hairpin ribozyme lacking a substrate moiety, comprising at least six bases in helix 2 and able to base-pair with a separate substrate RNA, wherein the said ribozyme comprises one or more bases 3' of helix 3 able to base-pair with the said substrate RNA to form a helix 5 and wherein the said ribozyme can cleave and/or ligate said separate RNA(s) in *trans*.
- 15 74. The ribozyme of claim 73, wherein said ribozyme comprises six bases in helix 2.
- 20 75. The ribozyme of claim 73, having the structure of Fig. 3, wherein each N and N' is independently any base and each dash may represent a hydrogen bond, r is 1-20, q is 2-20, o is 0 - 20, n is 1 - 4, and m is 1 - 20.
- 25 76. Method for increasing the activity of a hairpin ribozyme by providing one or more bases 3' of helix 3 able to base-pair with a substrate RNA to form a helix 5.
77. Trans-cleaving Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
78. Trans-ligating Hairpin ribozyme comprising at least 6 base pairs in helix 2 lacking a substrate RNA moiety.
79. The ribozyme of claim 73 having the structure of Fig. 73.
80. The ribozyme of claim 73 having the structure of Fig. 74.
- 30 81. A cell including the ribozyme of any of claims 73-80.

82. An expression vector comprising nucleic acid encoding the ribozyme of any of claims 73-80, in a manner which allows expression of that ribozyme within a cell.
83. A cell including an expression vector of claim 82.
- 5 84. Method for altering in vivo the nucleotide base sequence of a naturally occurring mutant nucleic acid molecule, comprising the steps of:
- 10 contacting said nucleic acid molecule in vivo with an oligonucleotide or peptide nucleic acid able to form a duplex or triplex molecule with said nucleic acid molecule, wherein formation of said duplex or triplex molecule directly, or after nucleic acid repair *in vivo*, causes at least one base in said nucleic acid molecule to be chemically modified to functionally alter the nucleotide base sequence of said nucleic acid sequence.
- 15 85. The method of claim 84, wherein said oligonucleotide is of a length sufficient to activate dsRNA deaminase in vivo to cause conversion of an adenine base to inosine in an RNA molecule.
- 20 86. The method of claim 84, wherein said oligonucleotide comprises an enzymatic nucleic acid molecule which is active to chemically modify a base.
87. The method claim 84, wherein said nucleic acid molecule is DNA or RNA.
88. The method of claim 84, wherein said oligonucleotide comprises a chemical mutagen.
- 25 89. The method of claim 88, wherein said mutagen is nitrous acid.
90. The method of claim 84 wherein said oligonucleotide causes deamination of 5-methylcytosine to thymidine, cytosine to uracil, or adenine to inosine, or methylation of cytosine to 5-methylcytosine.
- 30 91. The method of claim 84, wherein an endogenous mammalian editing system is co-opted to cause said chemical modification.

92. Method for introduction of enzymatic nucleic acid into a cell or tissue, comprising the steps of;

5 providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of
10 RNA from said first nucleic acid under said conditions;

and contacting said complex with said cell or tissue under conditions in which said enzymatic nucleic acid molecule is produced in said cell or tissue.

- 15 93. Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

providing a complex of a first nucleic acid molecule encoding said desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from
20 said first nucleic acid under said conditions;

25 and contacting said complex with said cell or tissue under conditions in which said desired acid molecule is produced in said cell or tissue.

- 94 Method for introduction of a desired nucleic acid into a cell or tissue, comprising the steps of;

30 providing a complex of a first nucleic acid molecule encoding said enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired

structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions;

5 and wherein said second nucleic acid further comprises a localization factor;

and contacting said complex with said cell or tissue under conditions in which said desired nucleic acid molecule is produced in said cell or tissue.

10 95. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; 15 wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.

20 96. Complex of a first nucleic acid molecule encoding a desired nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said first nucleic acid molecule lacks a promoter region and said R-loop is formed in a region of said first nucleic acid 25 molecule at a location which promotes expression of RNA from said first nucleic acid under said conditions.

30 97. Complex of a first nucleic acid molecule encoding an enzymatic nucleic acid associated with a second nucleic acid molecule having sufficient complementarity with said first nucleic acid molecule so that it is able to form an R-loop base-paired structure under physiological conditions with said first nucleic acid molecule; wherein said R-loop is formed in a region of said first nucleic acid molecule at a location which promotes expression of RNA from said

first nucleic acid under said conditions, and wherein said second nucleic acid further comprises a localization factor.

:

:

:

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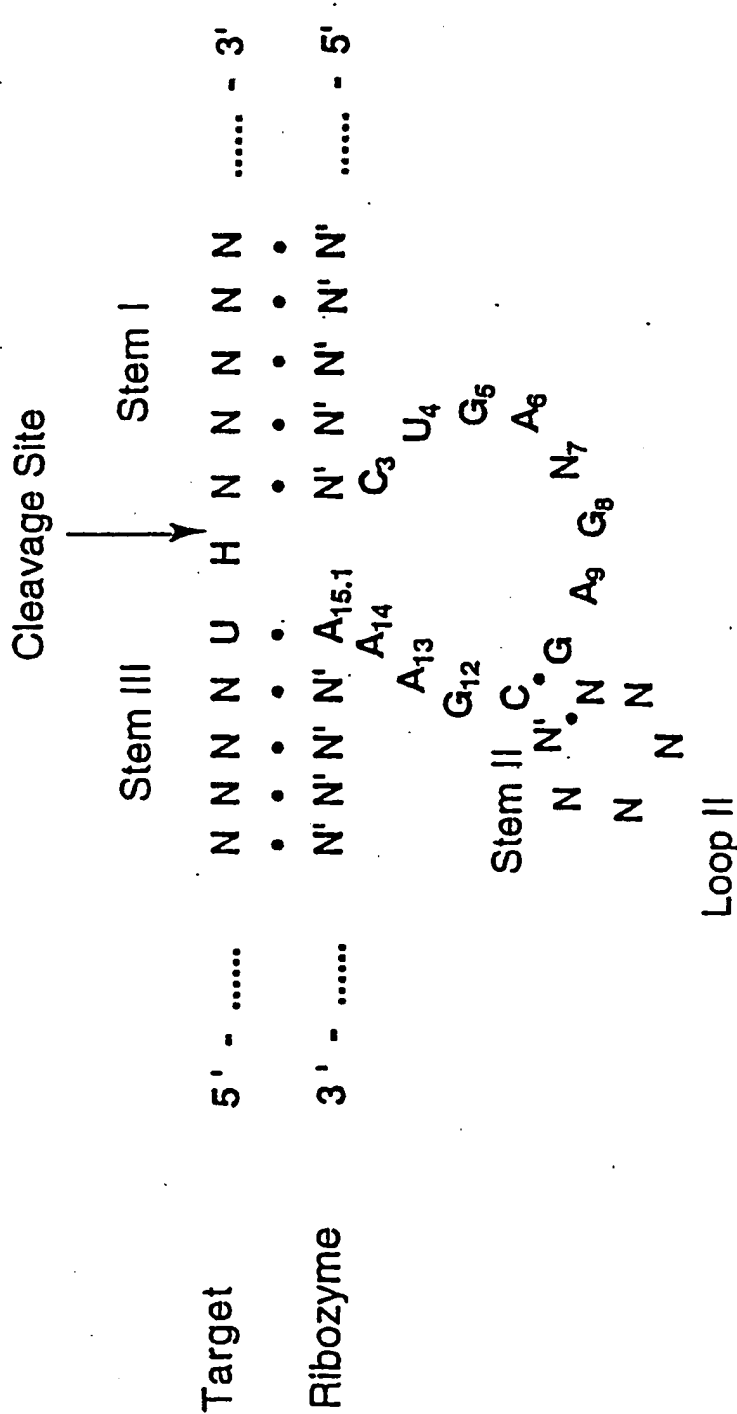
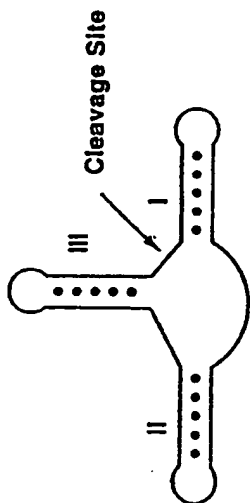


FIG. 1.

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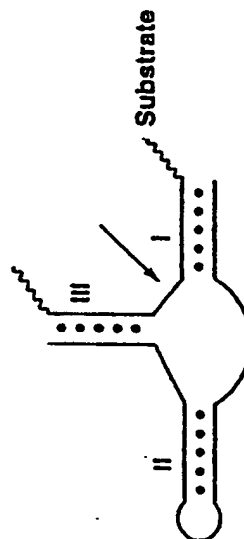
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FIG. 2a.



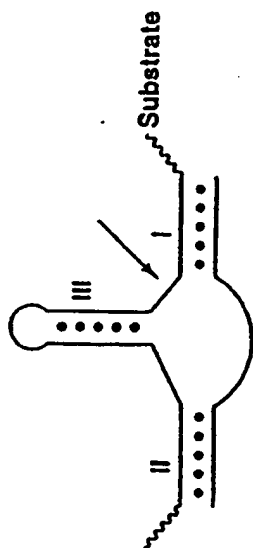
a

FIG. 2c.



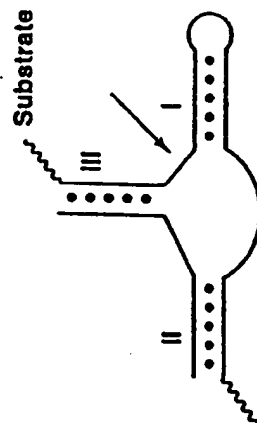
c

FIG. 2b.



b

FIG. 2d.



d

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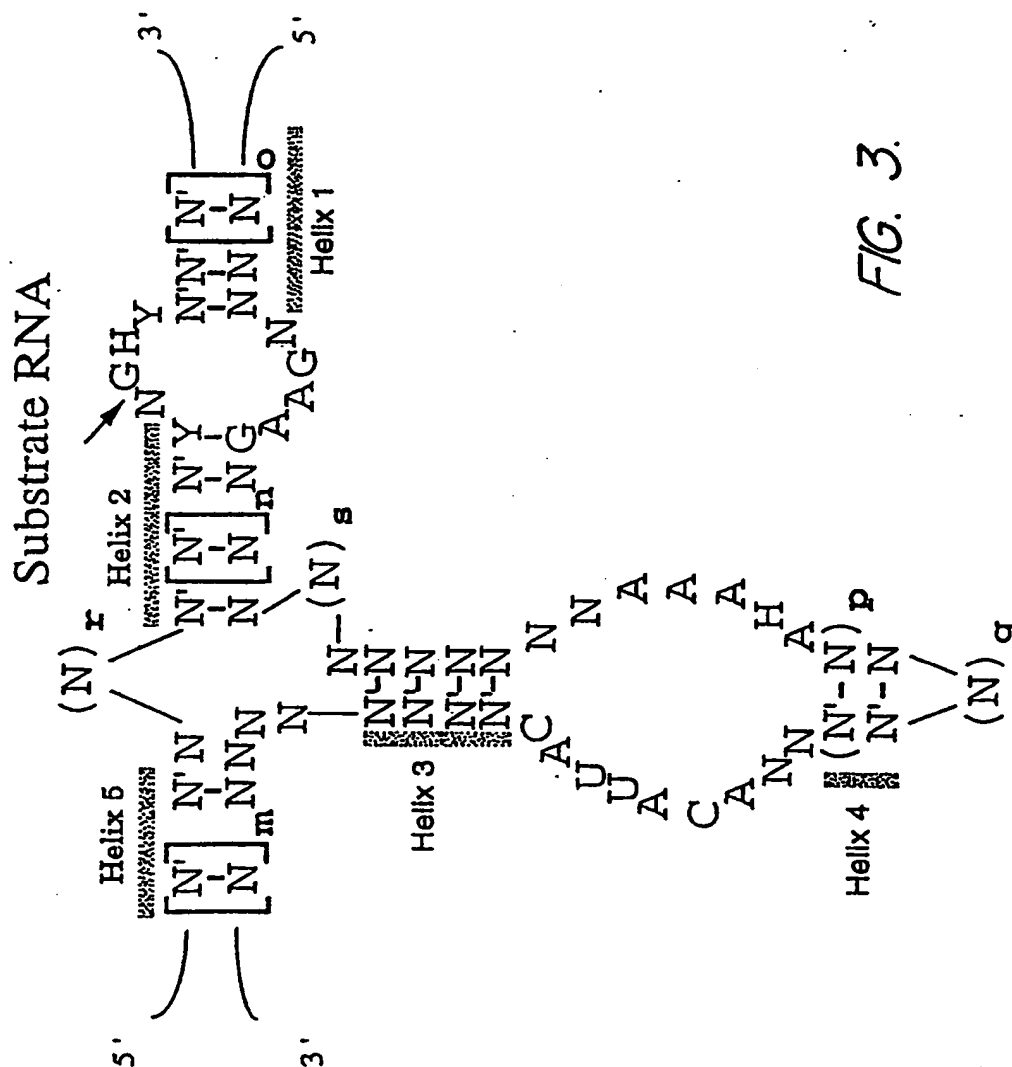
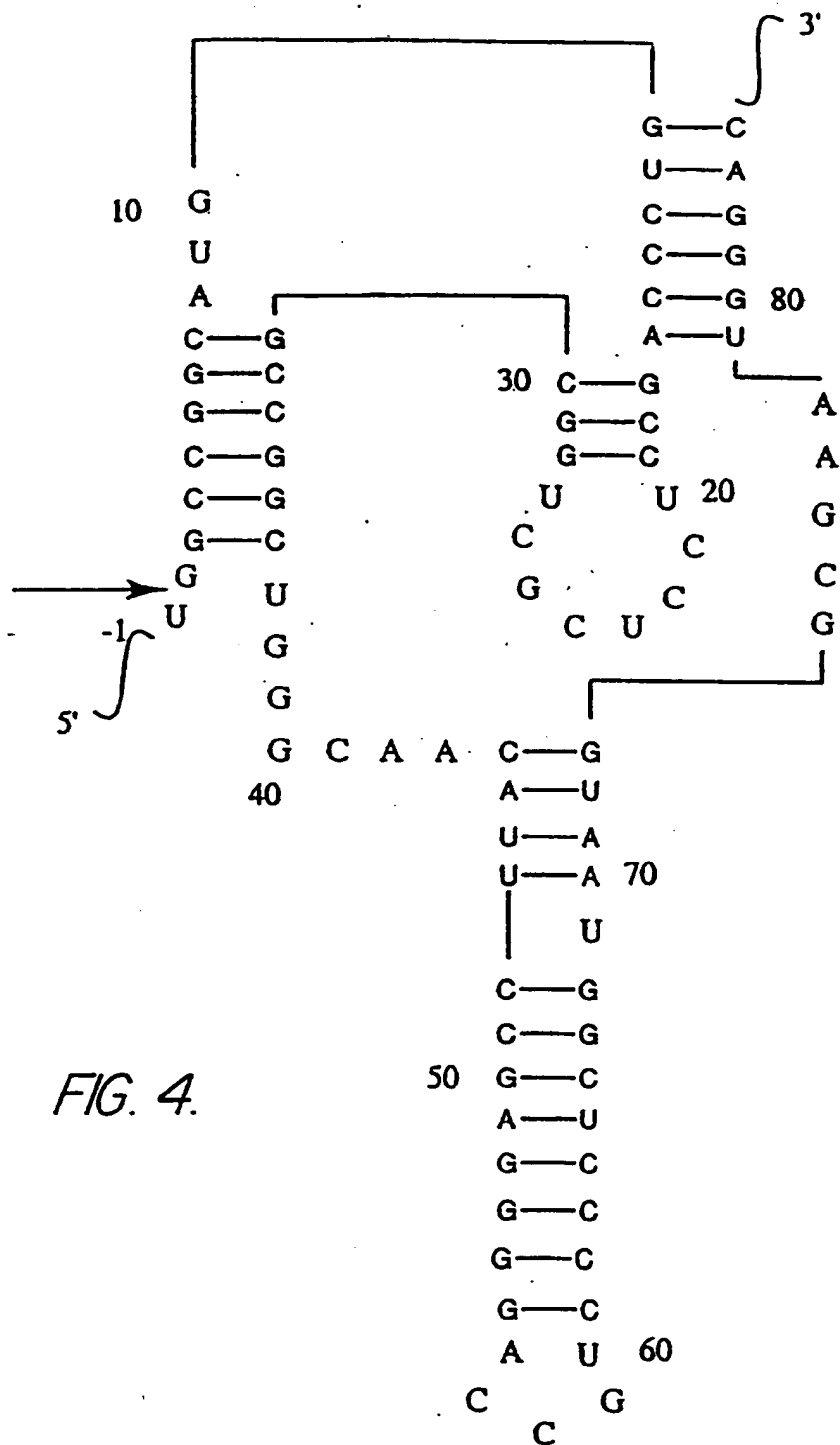


FIG. 3.

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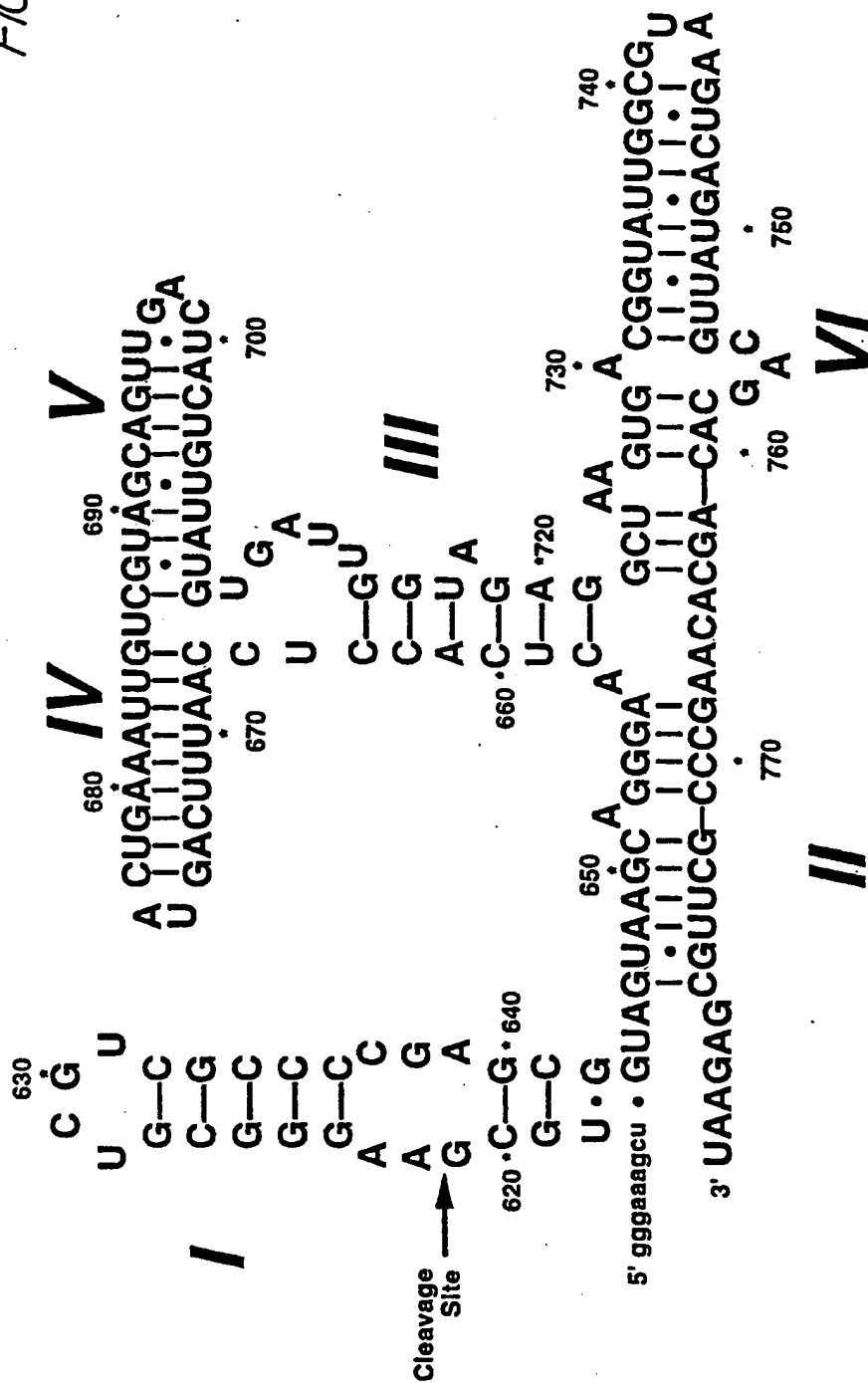


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NUC 37911

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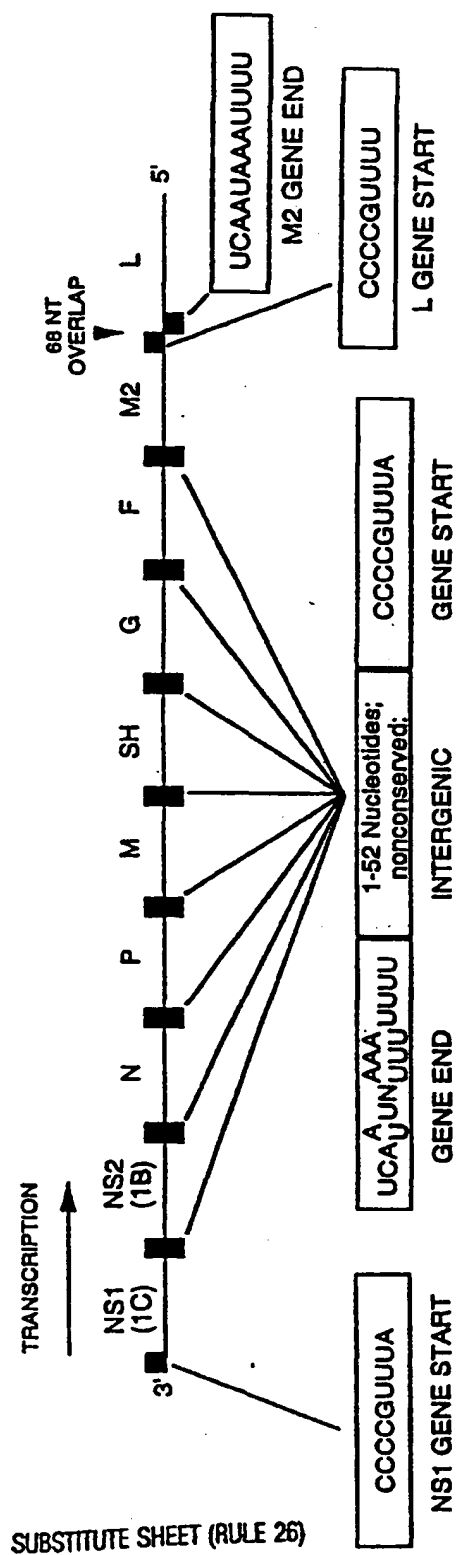
FIG. 5.



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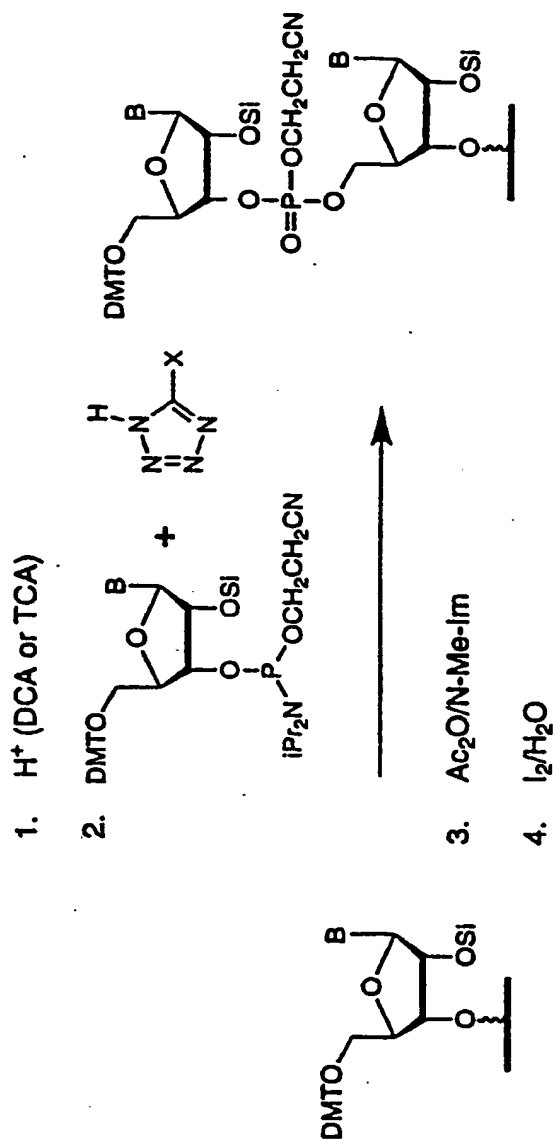
FIG. 6.



Adapted from Virology, Second Edition, Edited by B.N. Fields, 1990.

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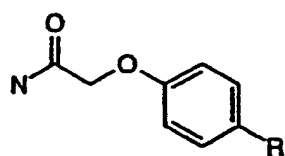
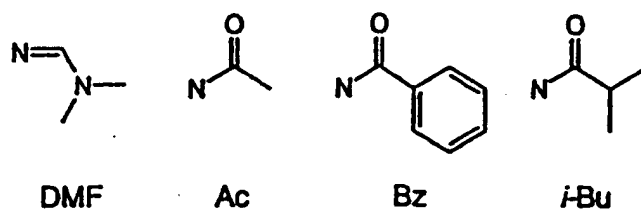
FIG. 7.



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*FIG. 8.*

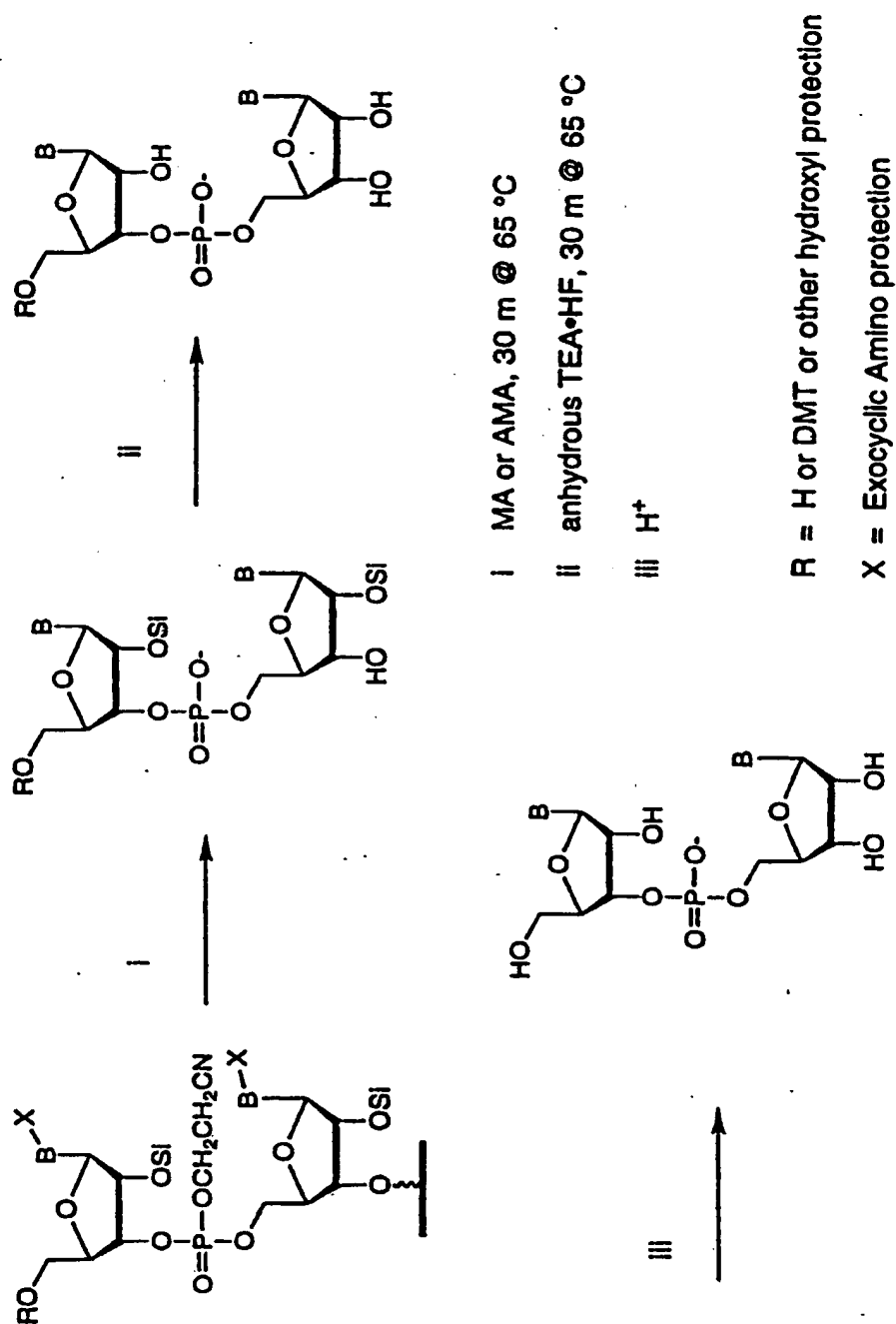
R = H = PAC
R = tBu = TAC
R = iPr = IPPAC

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FIG. 9.

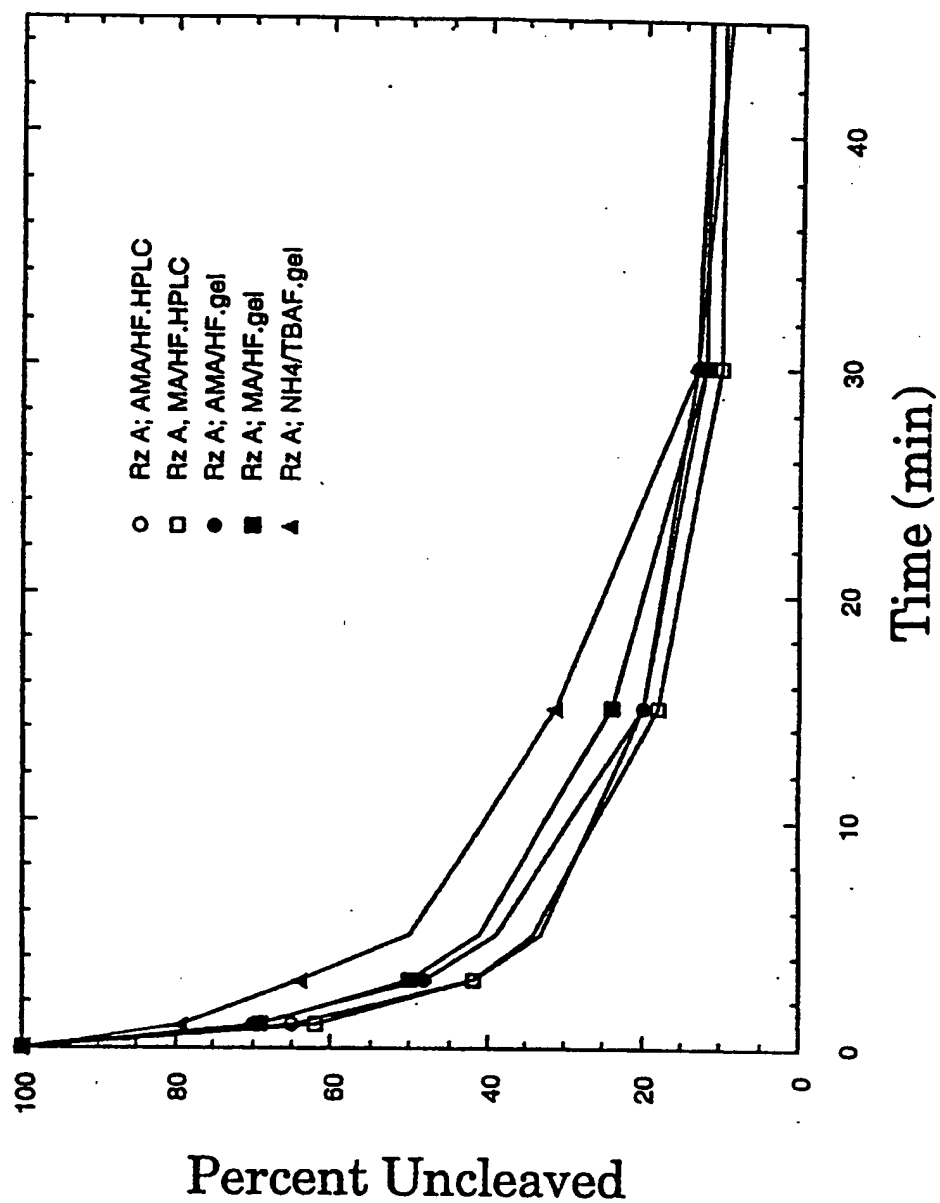


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FIG. 10.

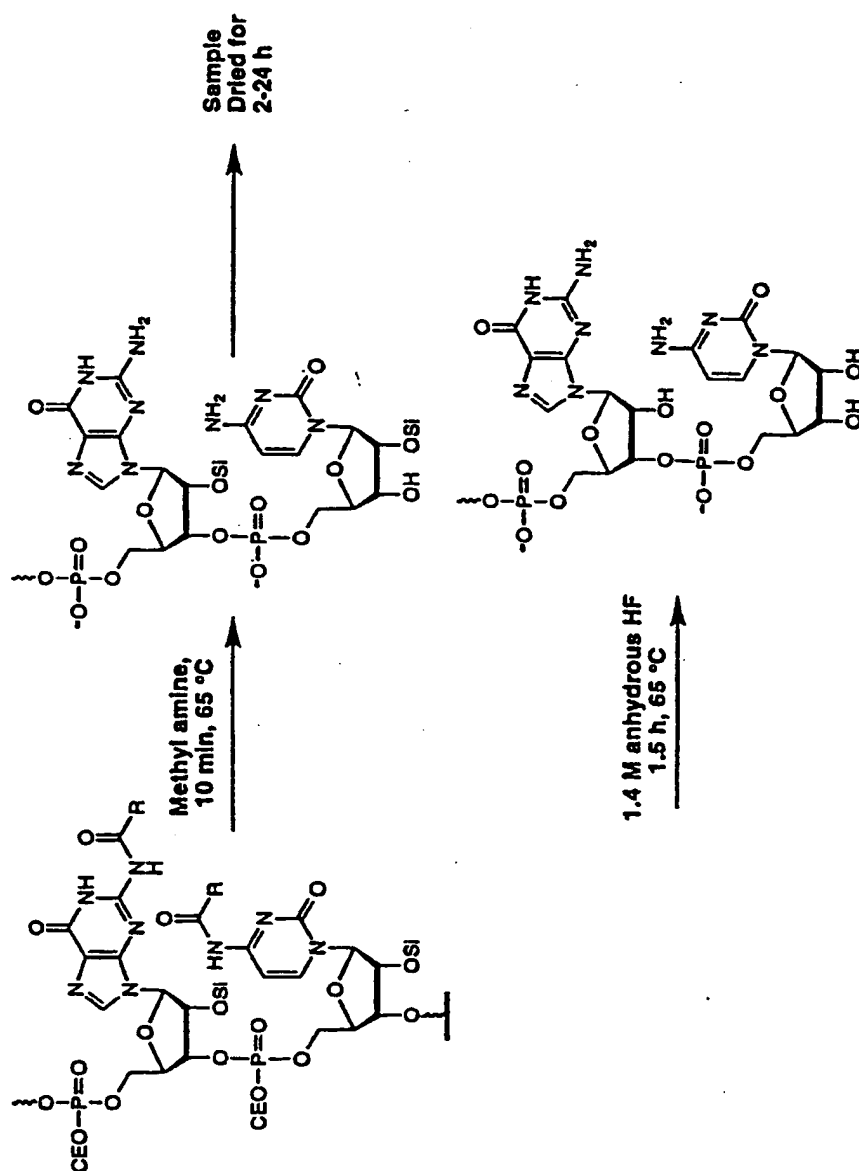


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FIG. 11.



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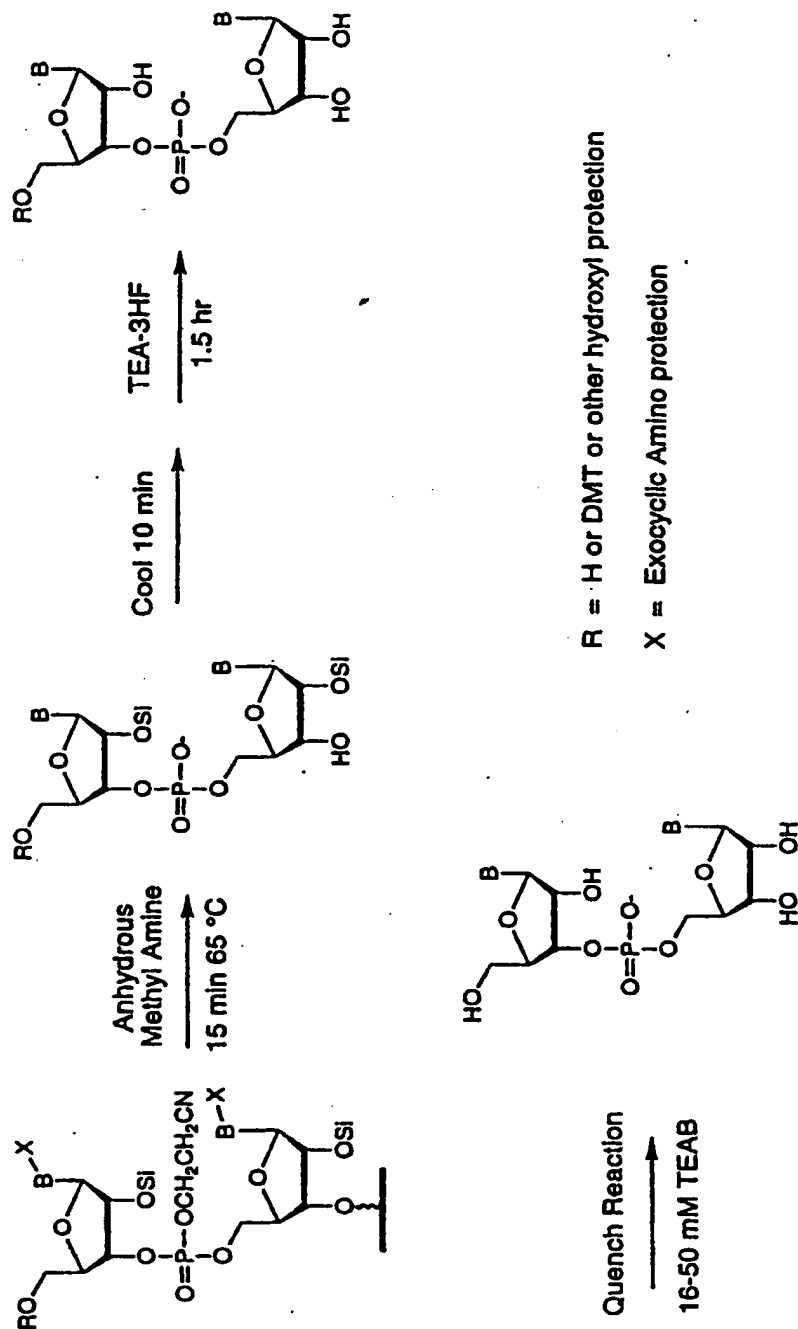


FIG. 12.

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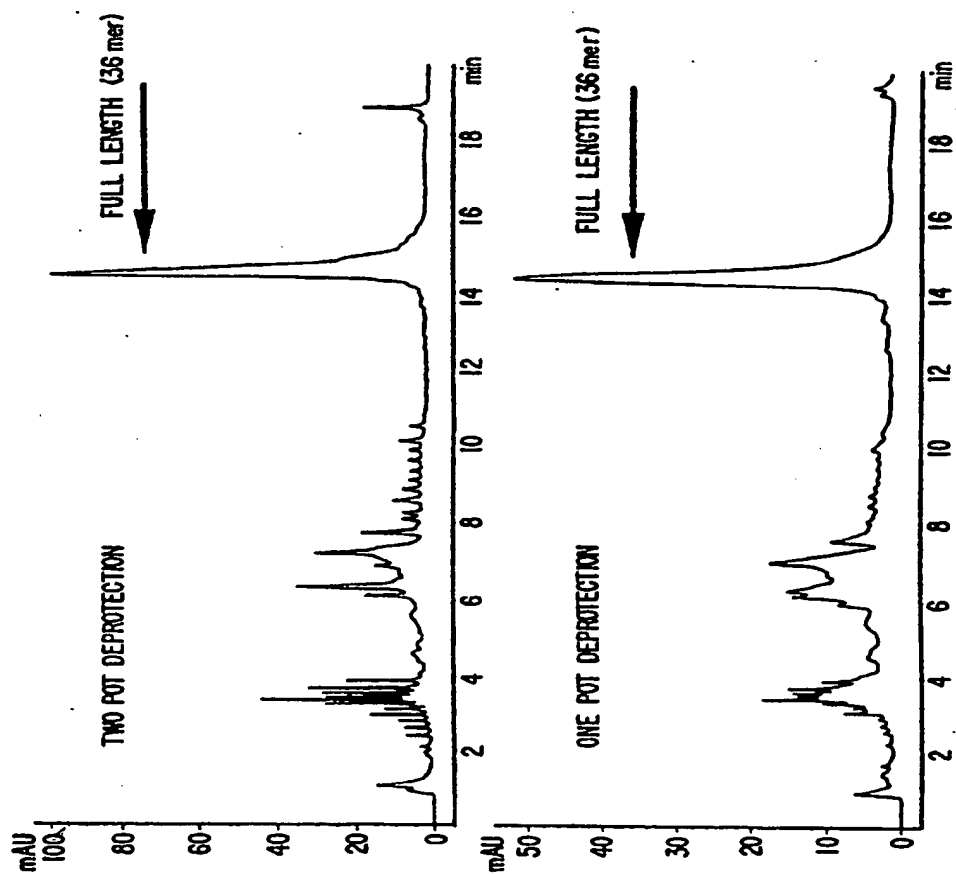


FIG. 13a.

FIG. 13b.

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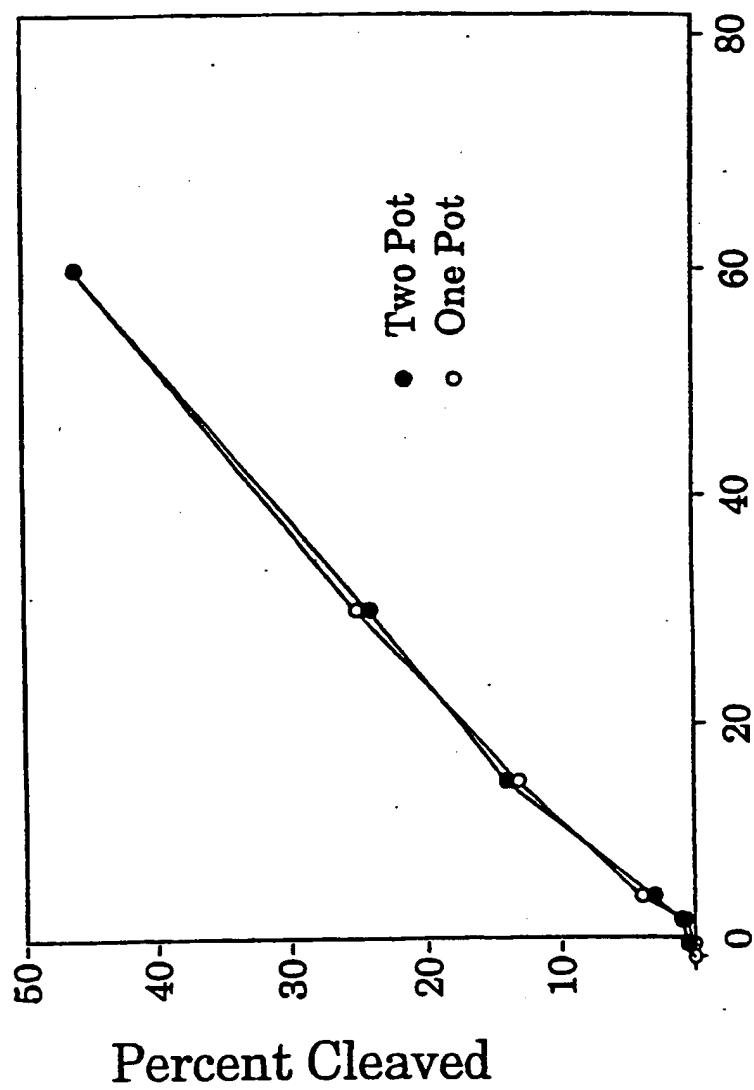


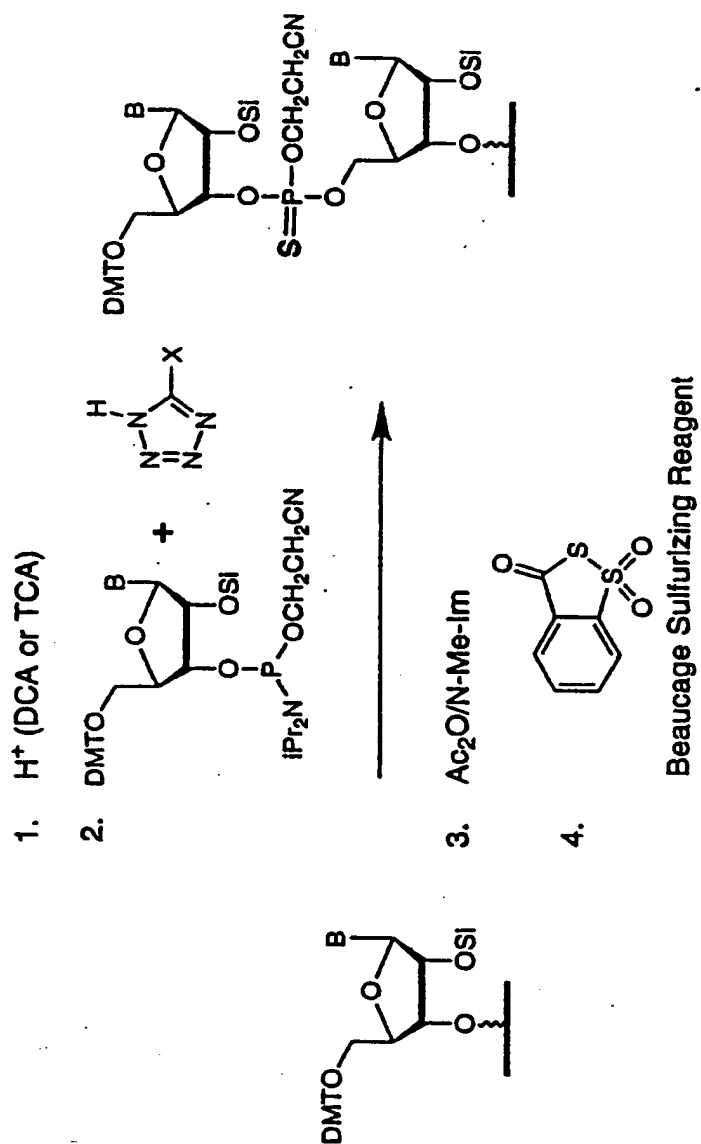
FIG. 14.

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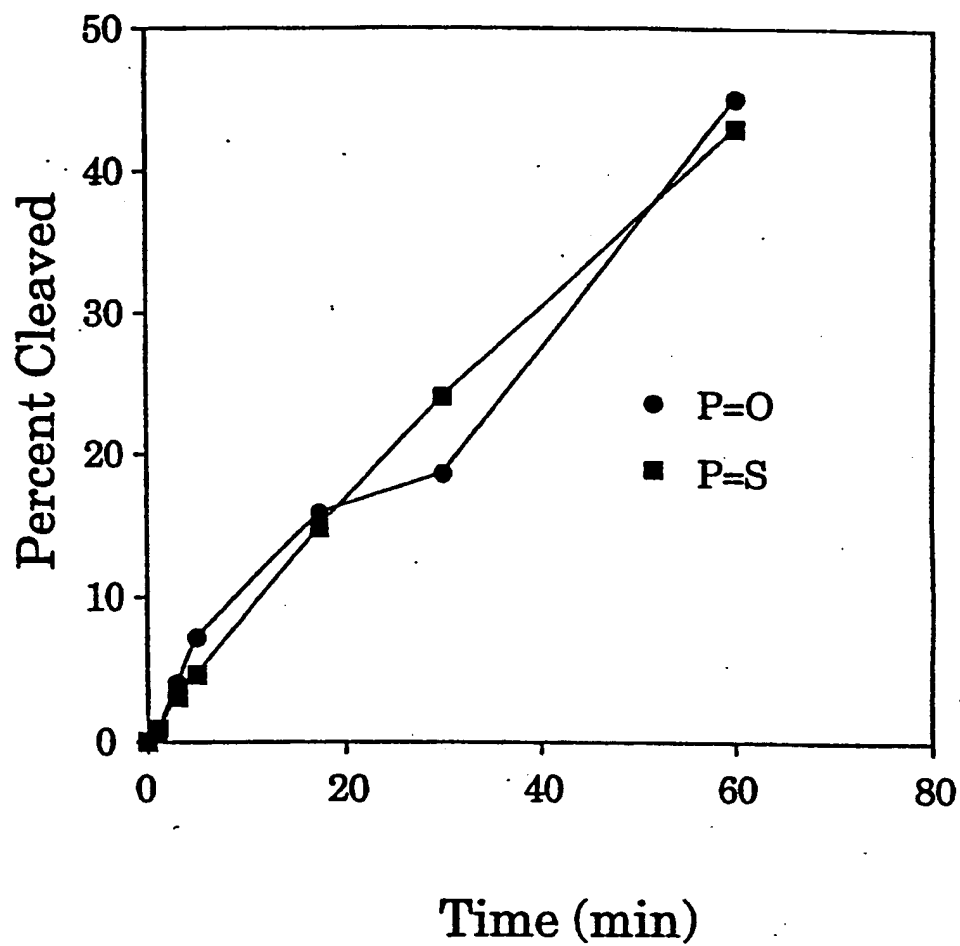
FIG. 15.



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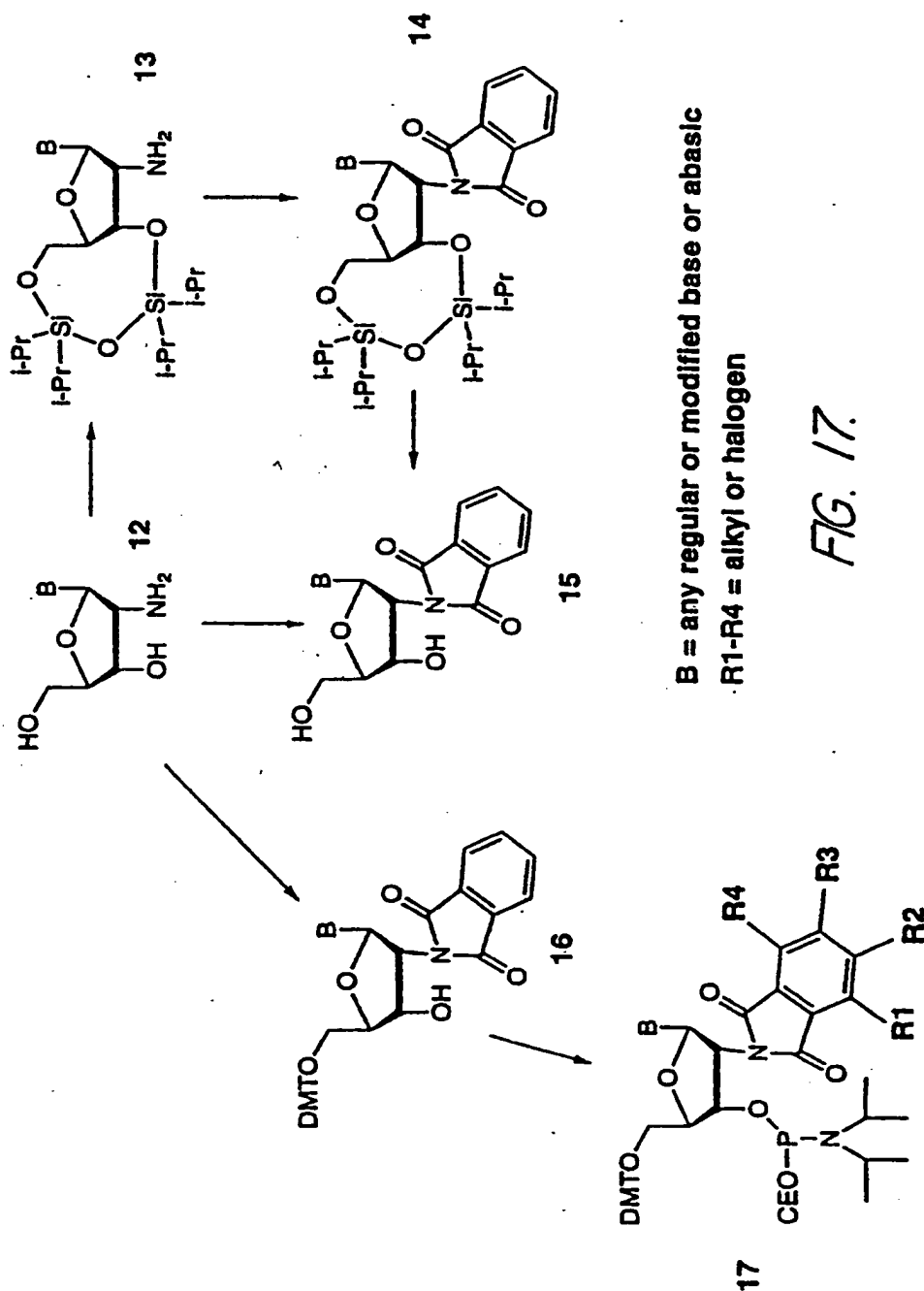


Time (min)
FIG. 16.

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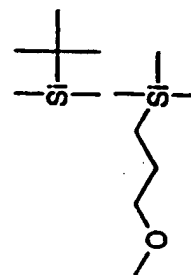
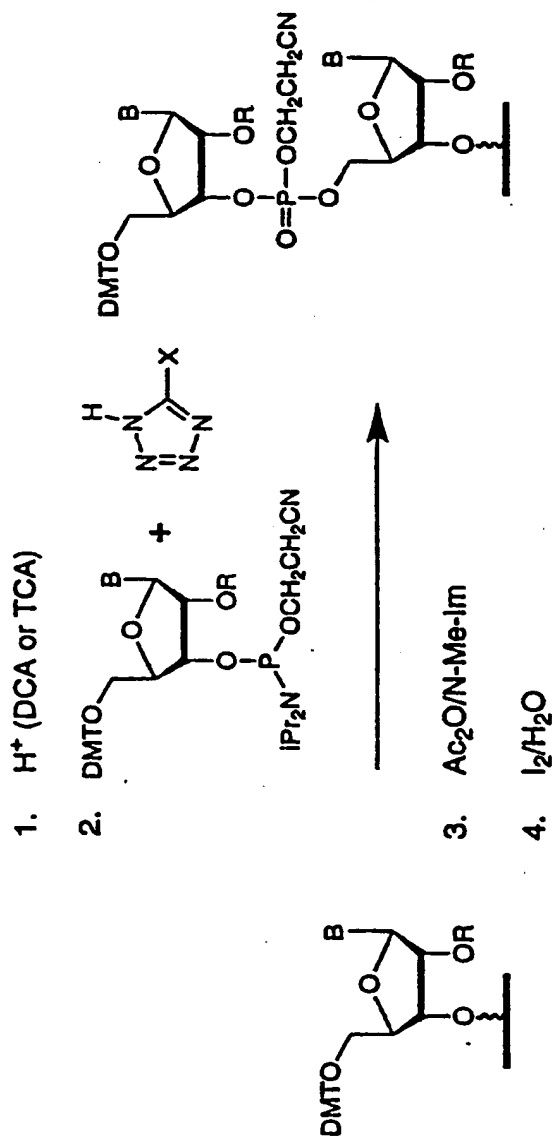


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FIG. 18.



R = Silyl ether (prior art)

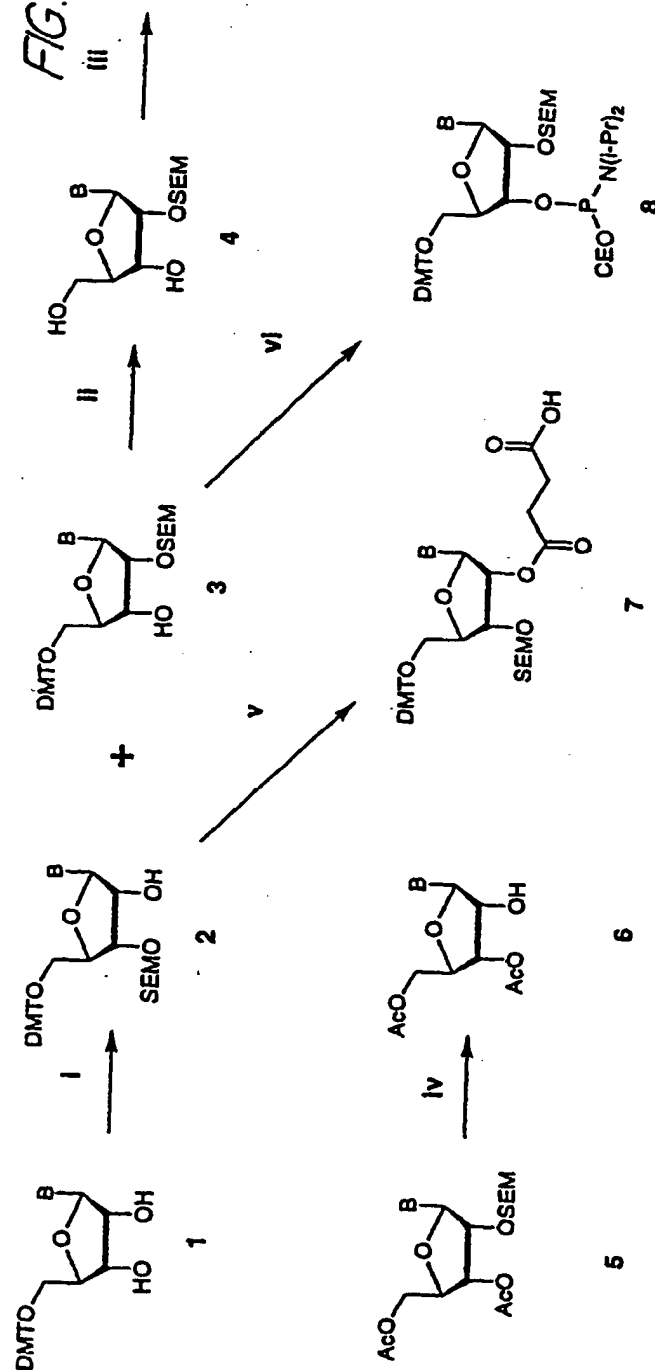
(trimethylsilyl)ethoxymethyl (SEM)

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FIG. 19.



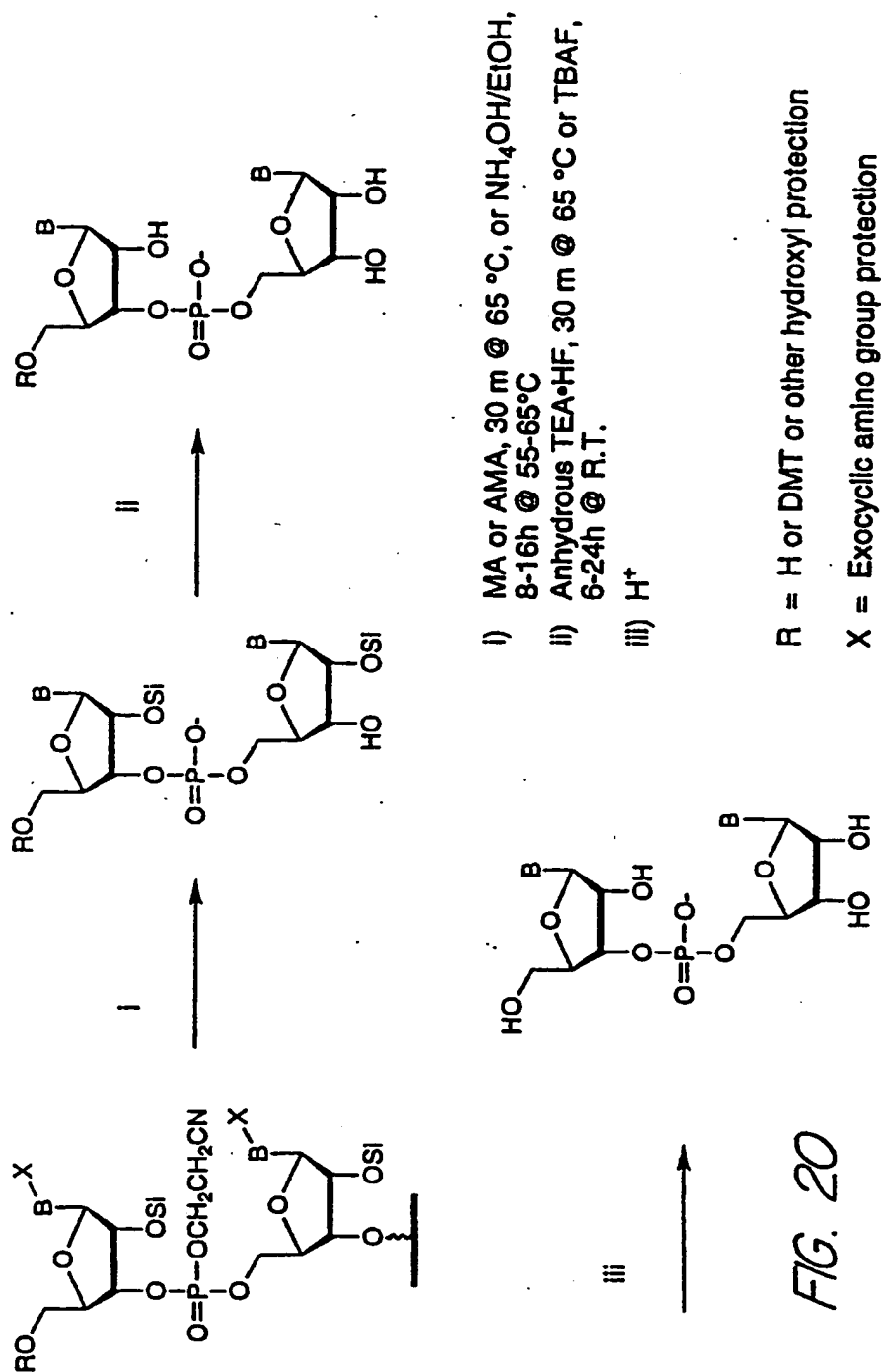
- I) = $\text{SnBu}_2\text{O}/\text{SEM-Cl}$
- II) = H^+
- III) = Ac_2O
- IV) = $\text{BF}_3 \cdot \text{OEt}_2$
- V) = Succinic Anhydride
- VI) = $\text{P}(\text{OCE})(\text{N-IPr}_2)\text{Cl}$

B = Protected A, C, G, U, T, 2AP, I, DIAP, P etc.

SEM = (trimethylsilyl)ethoxymethyl

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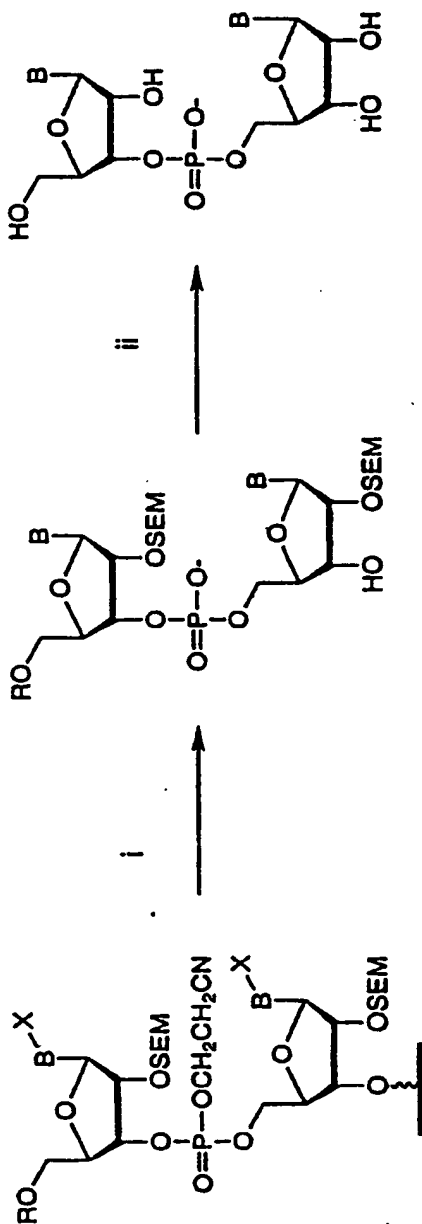
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FIG. 21.

i) MA or AMA, 30 m @ 65 °C or NH₄OH or NH₄OH/EtOH, 8-16h @ 55-65°Cii) BF₃•OEt₂

SEM = (trimethylsilyl)ethoxymethyl

R = H or DMT or other hydroxyl protection

X = Exocyclic amino group protection

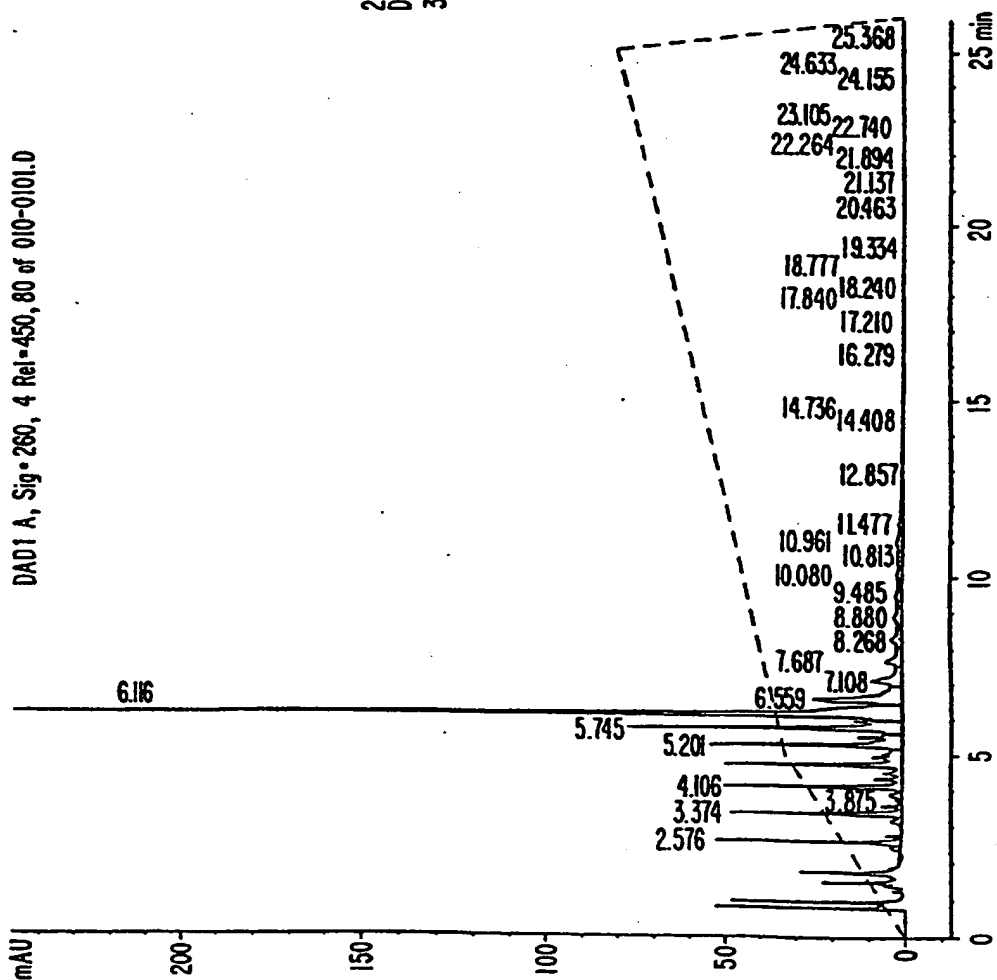
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FIG. 22.

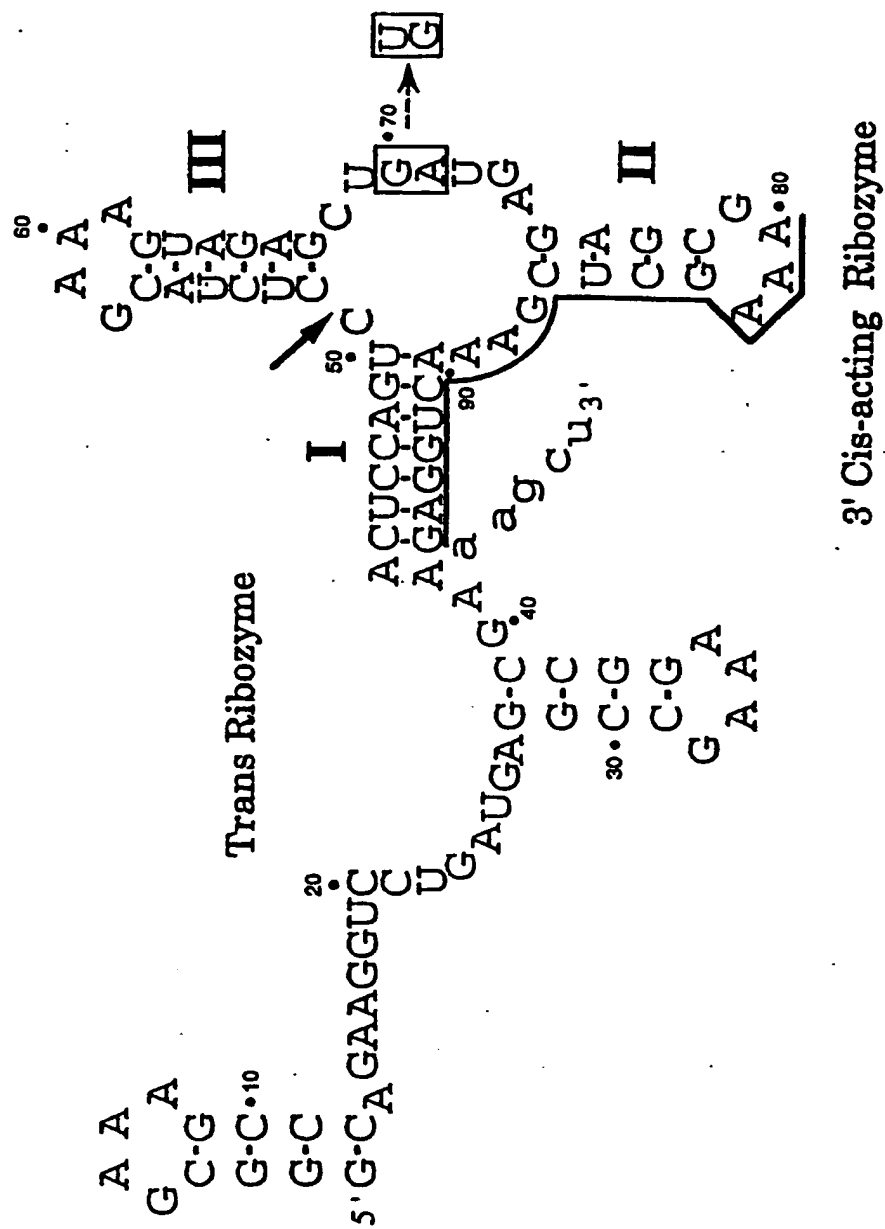
2'-O-SEM PROTECTED U¹⁰-mer
DEPROTECTED WITH BF₃·OEt₂
30m, 3eq./nucleotide



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FIG. 23.



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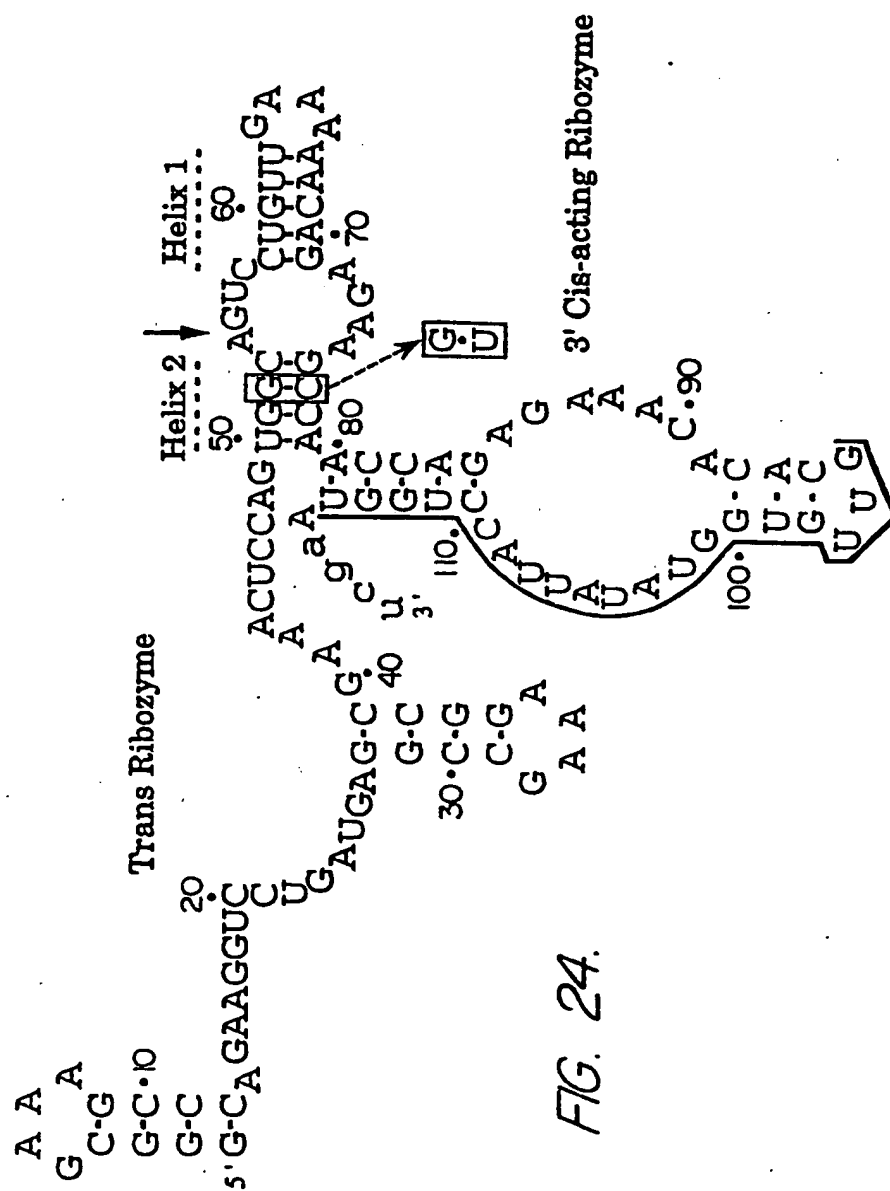


FIG. 24.

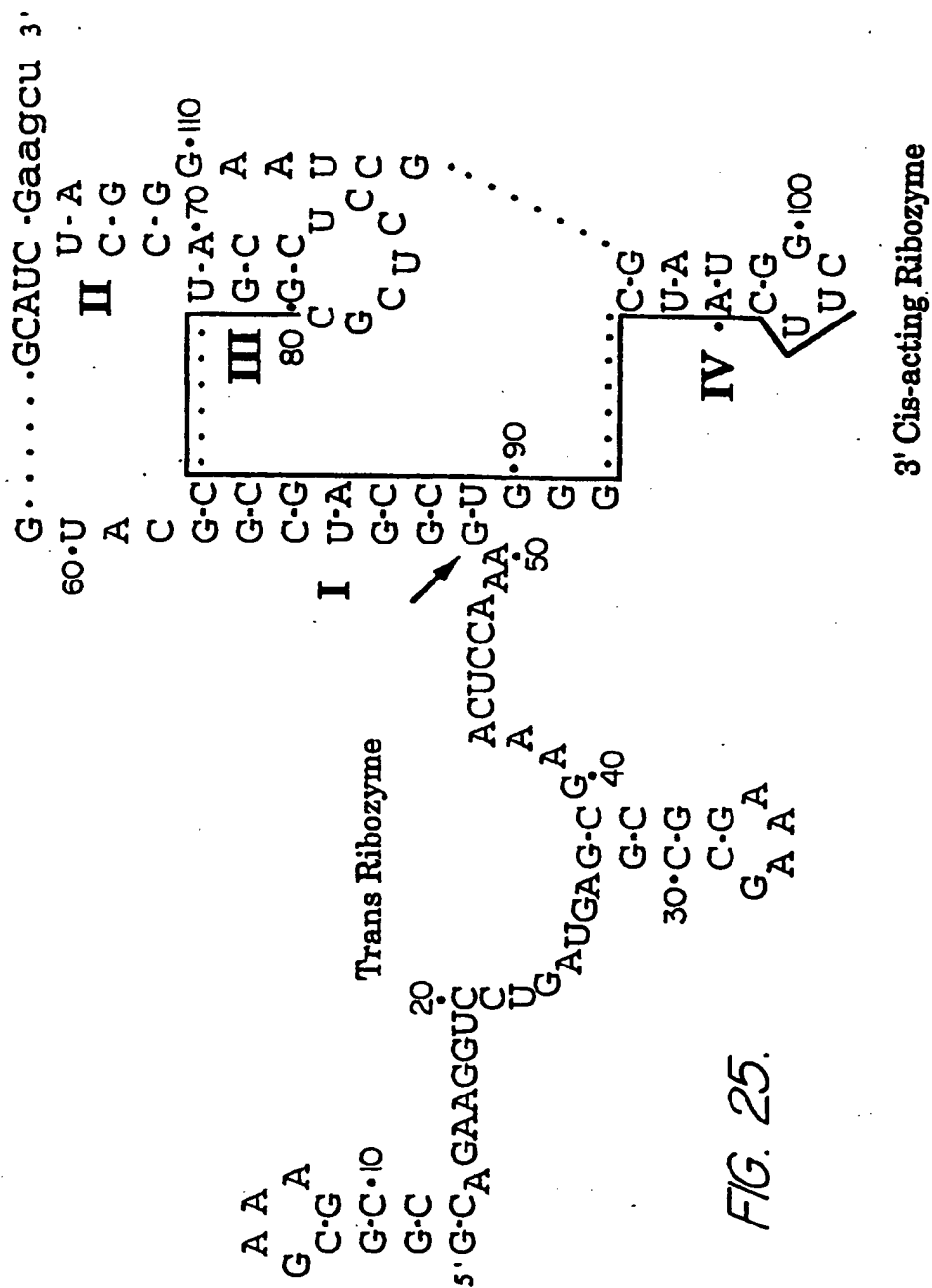
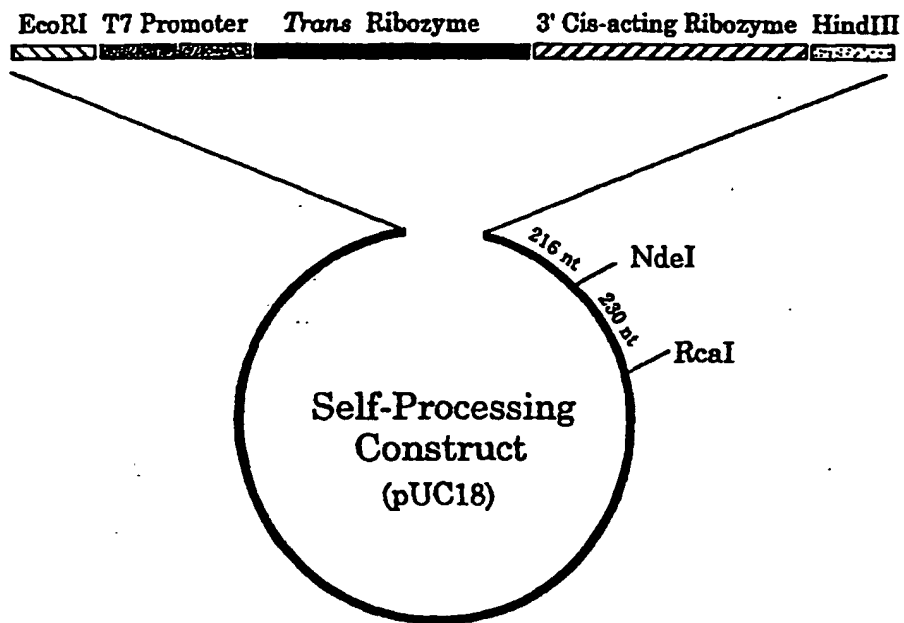


FIG. 25.

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FIG. 26.

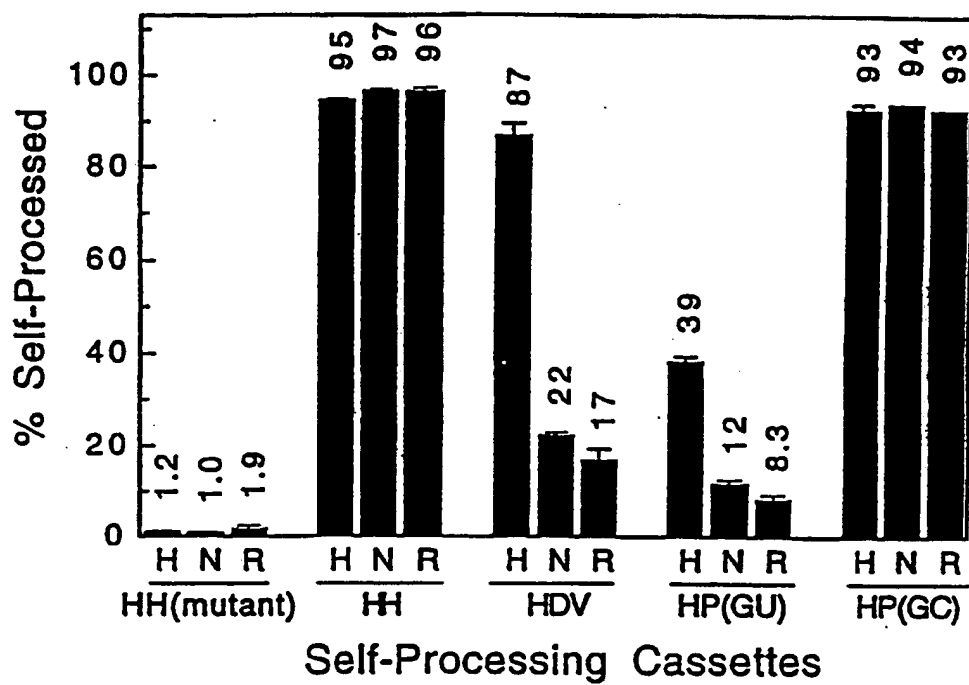


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FIG. 27.



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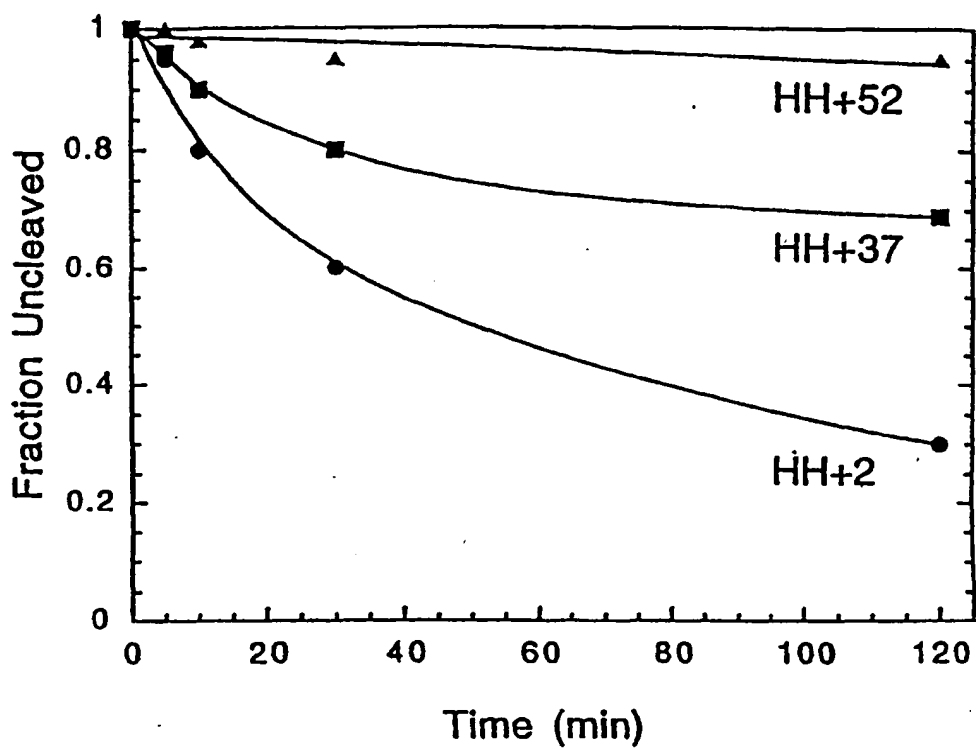


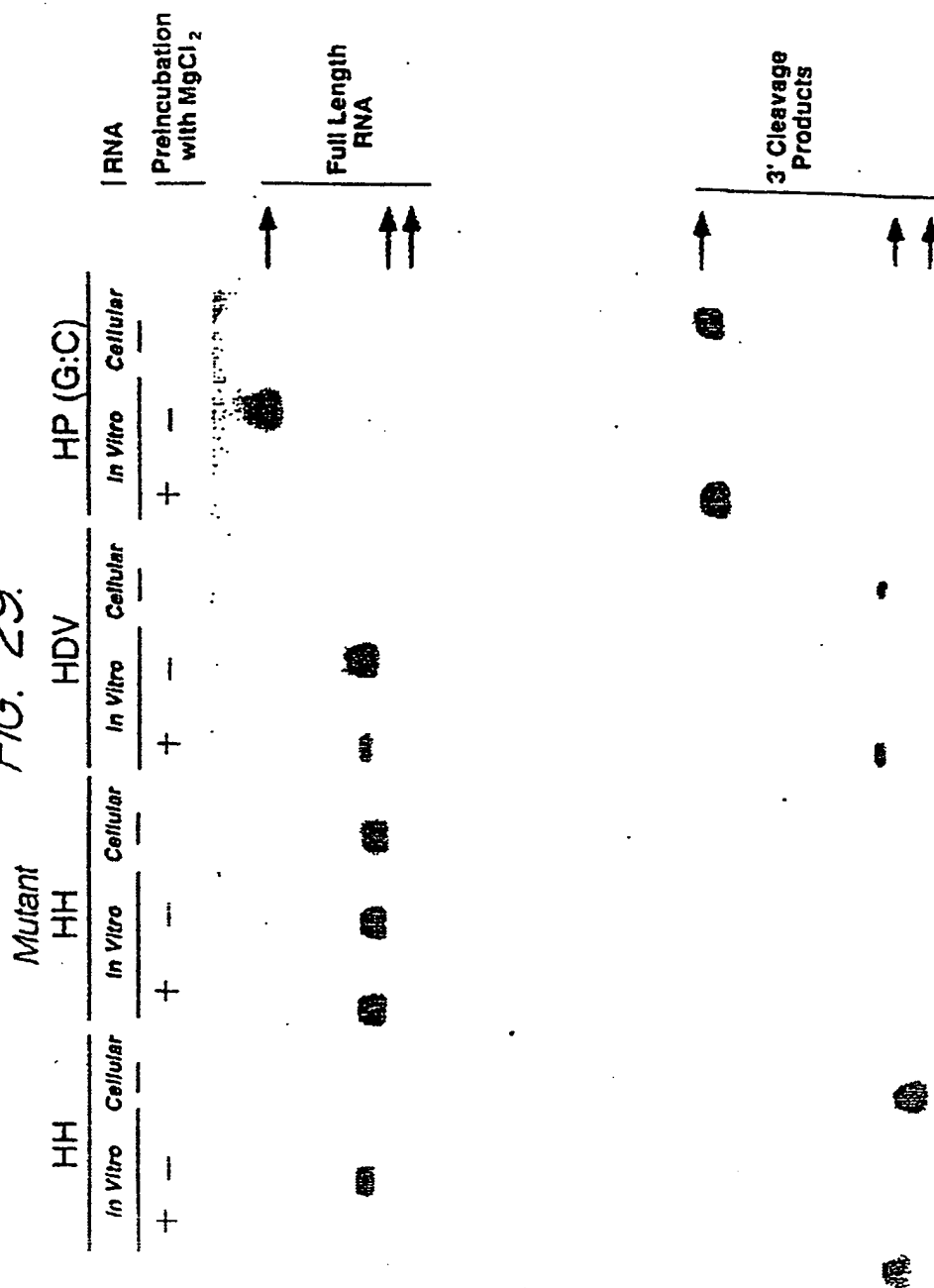
FIG. 28.

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FIG. 29.



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FIG. 30

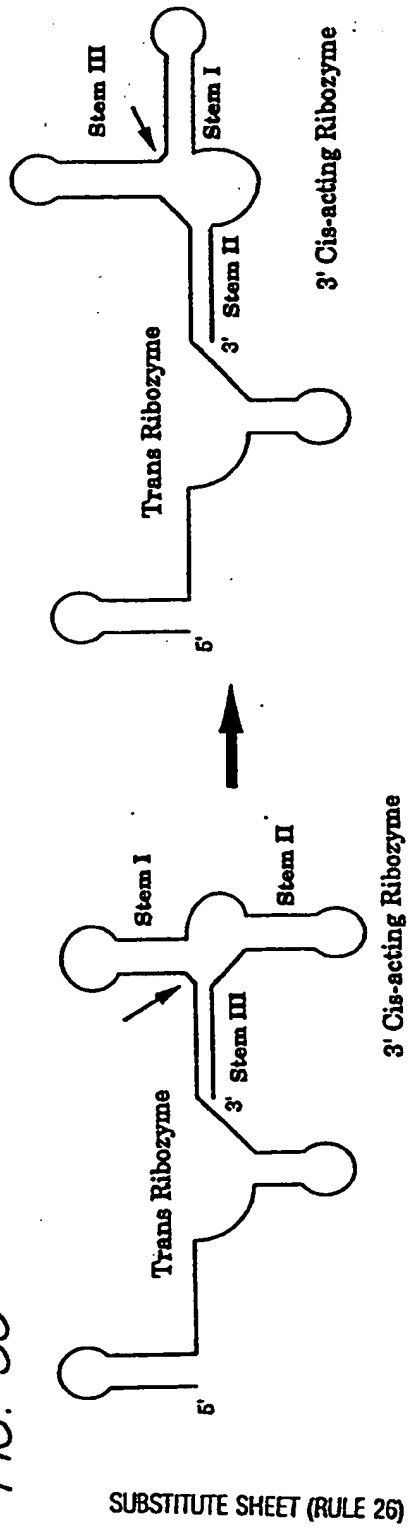
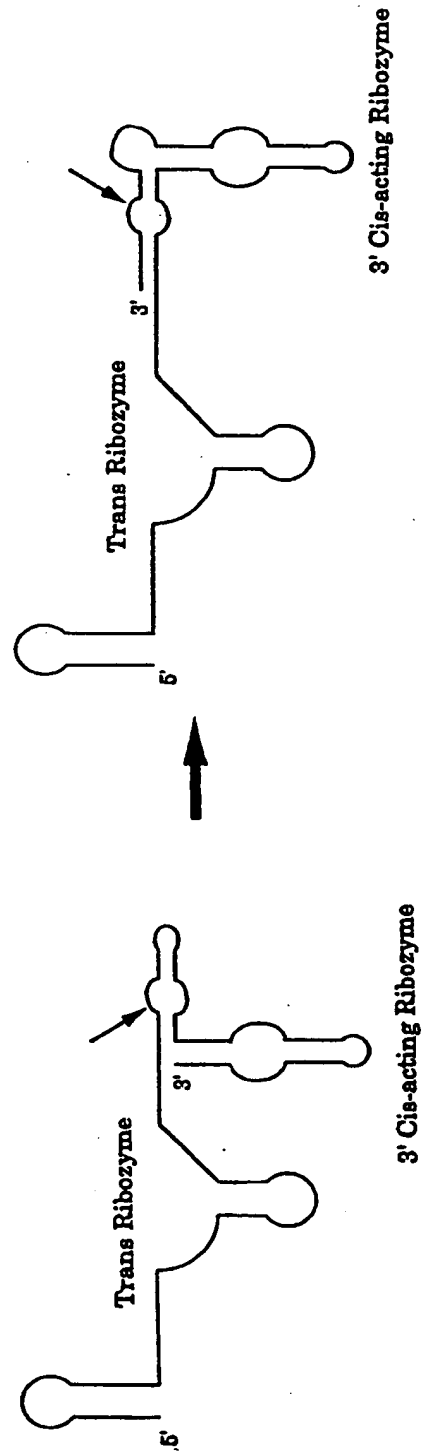


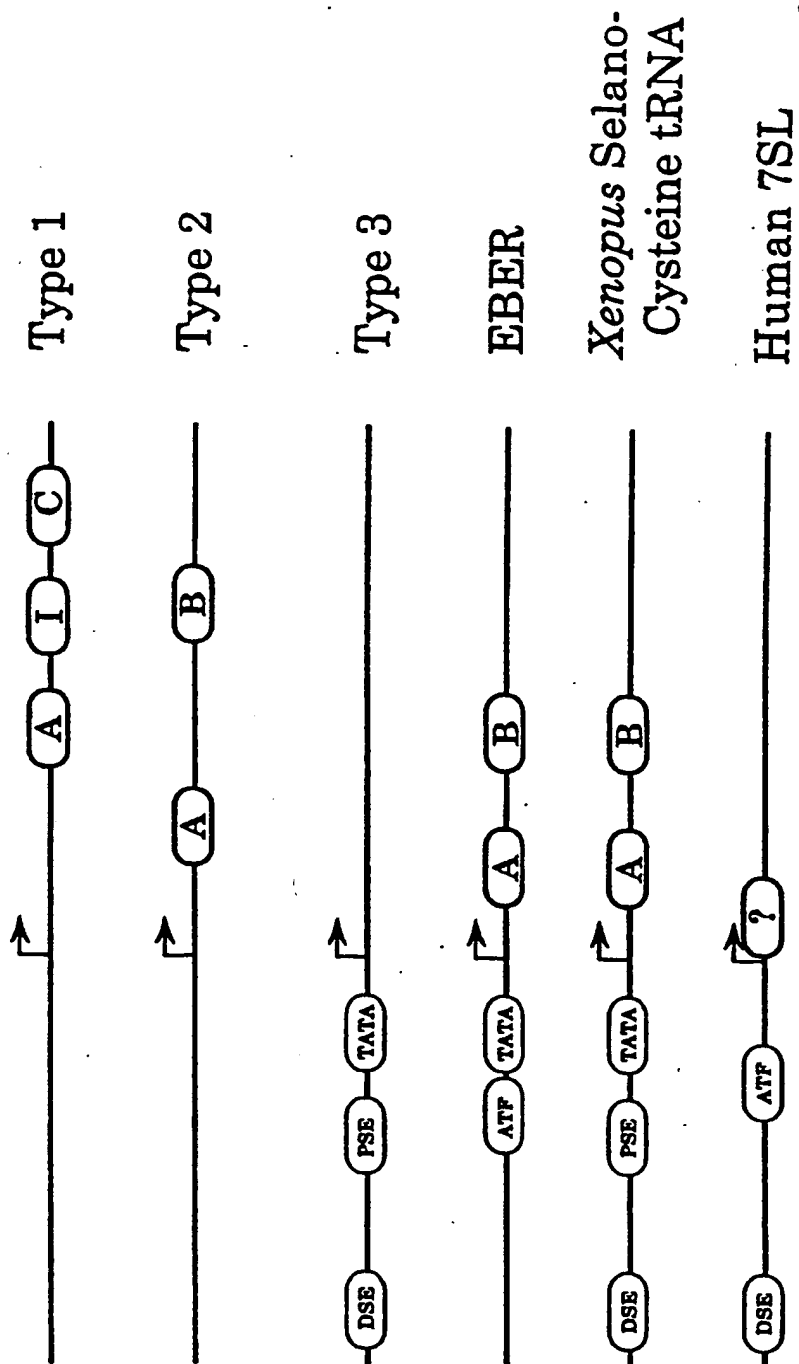
FIG. 31.



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FIG. 32.



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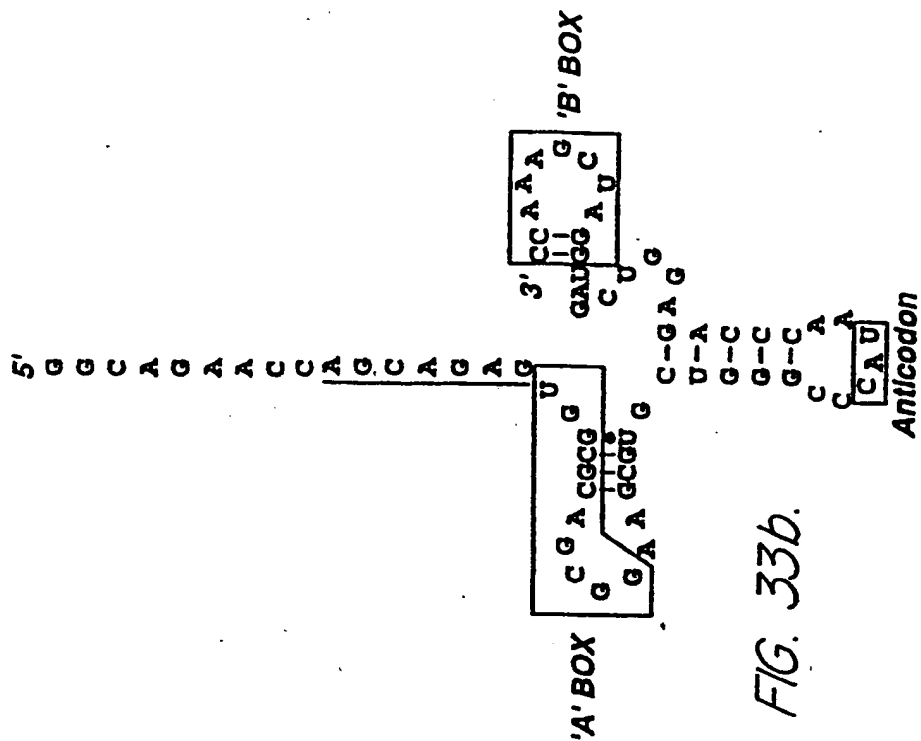


FIG. 33b.

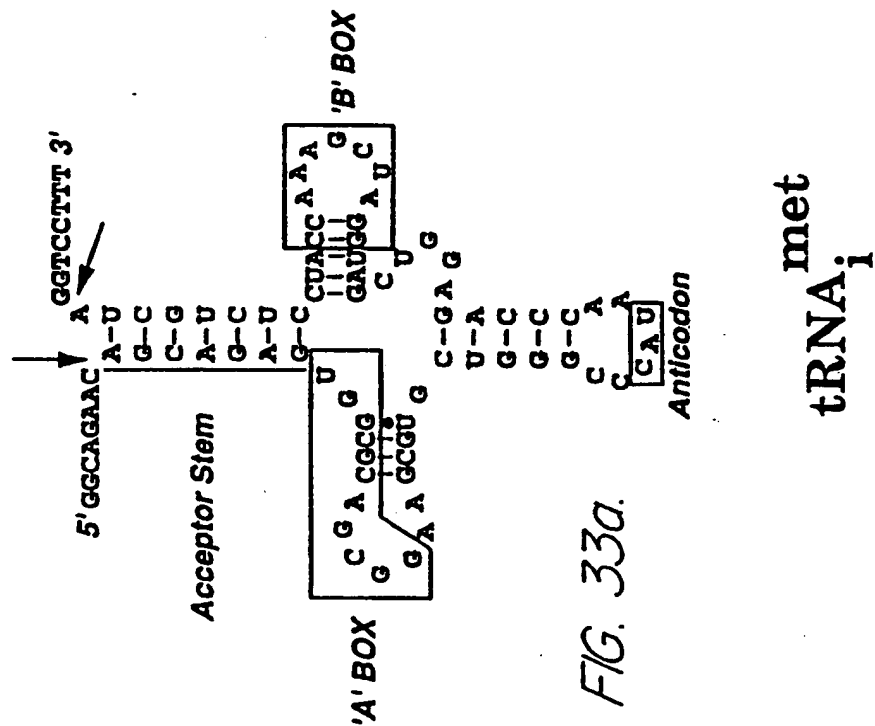


FIG. 33a.

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FIG. 34a.
 $\Delta 3-5$ /HHI

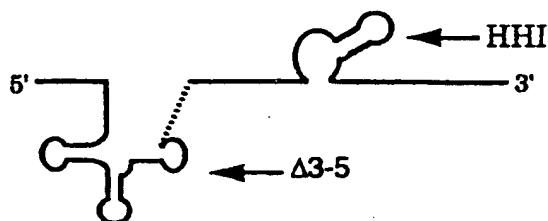


FIG. 34b.
S3

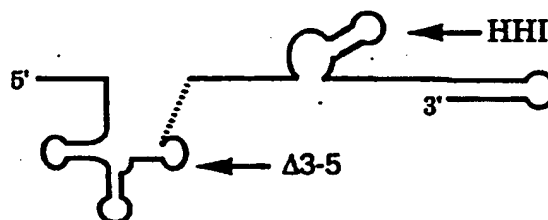


FIG. 34c.
S5

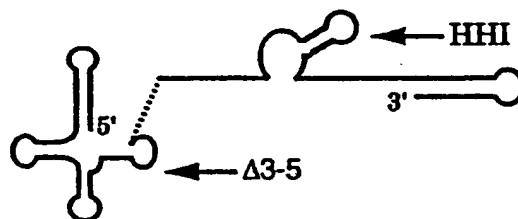


FIG. 34d.
S35

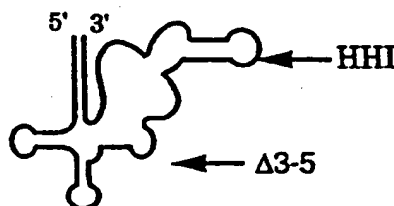
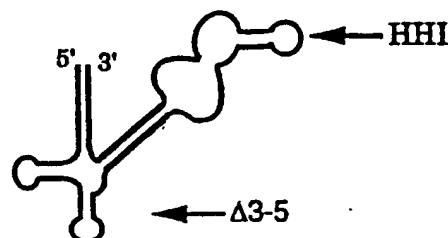


FIG. 34e.
S35Plus



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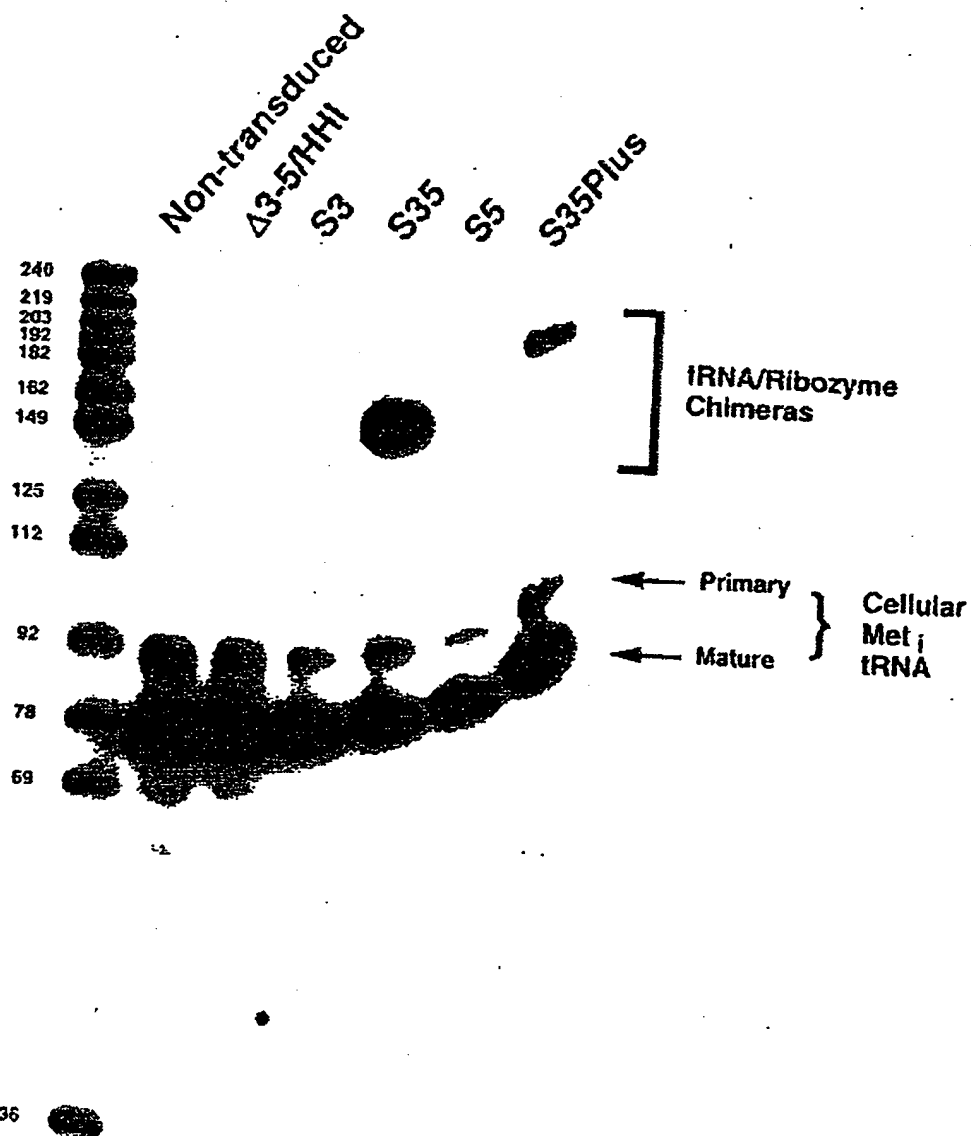


FIG. 35.

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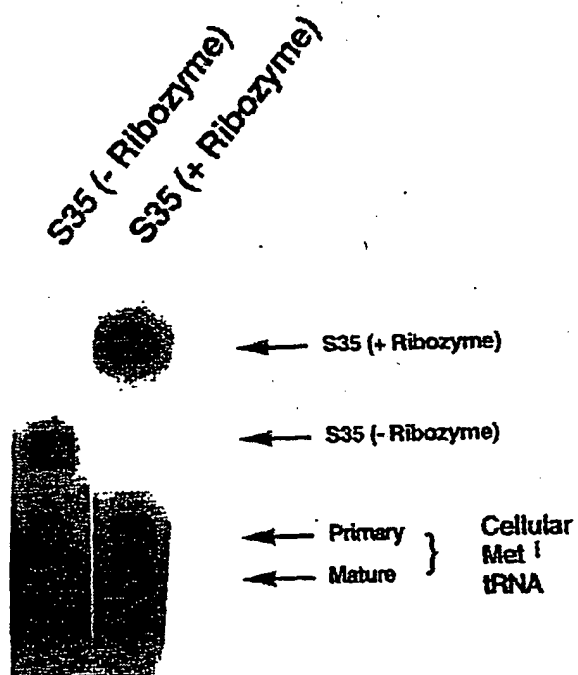


FIG. 36.

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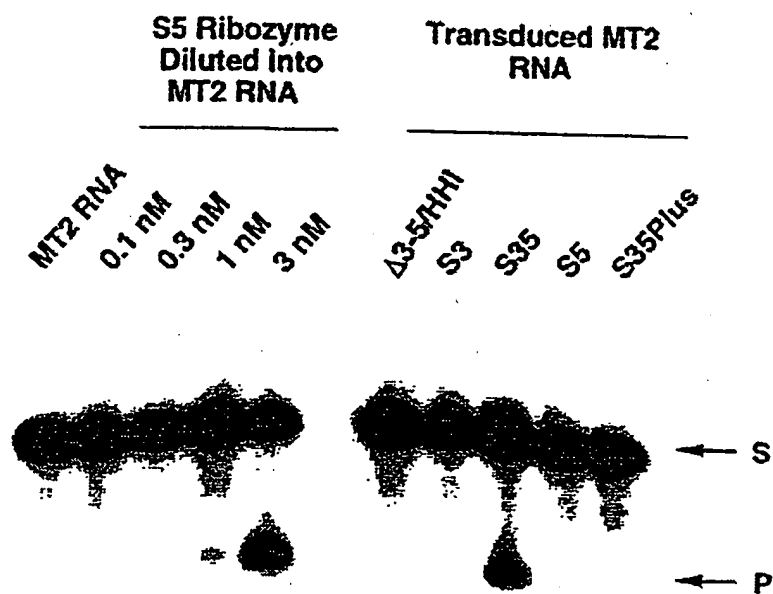
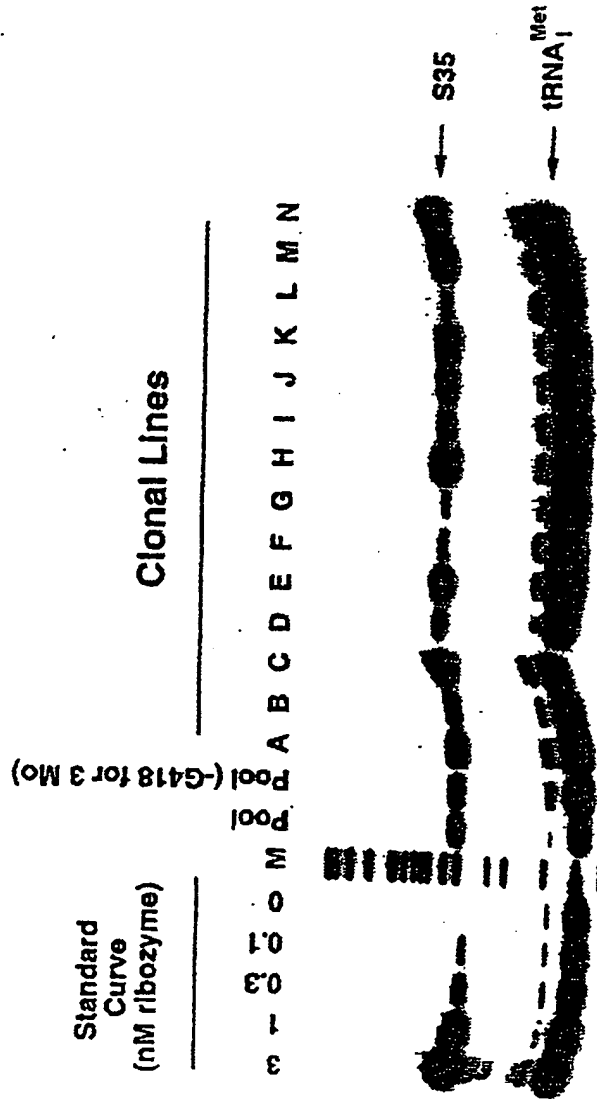


FIG. 37.

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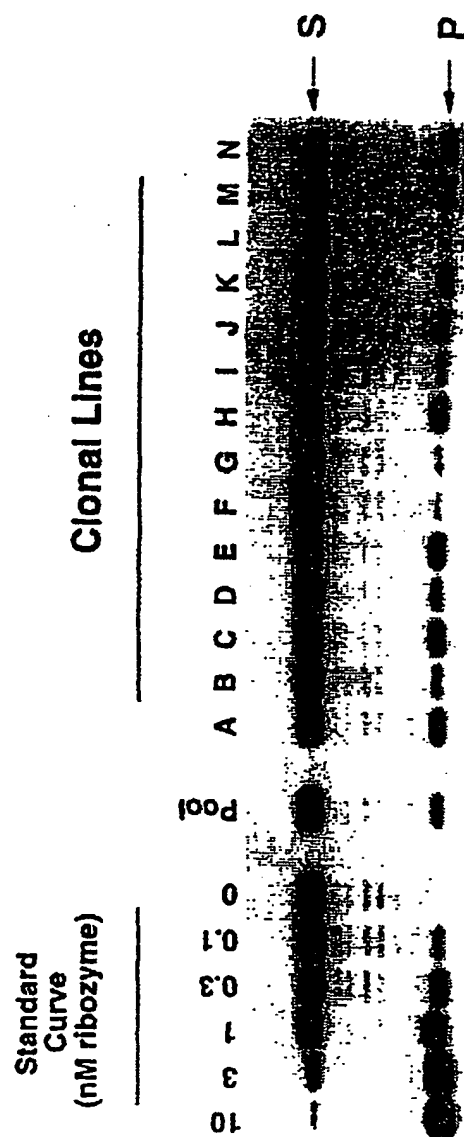
FIG. 38



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FIG. 39.



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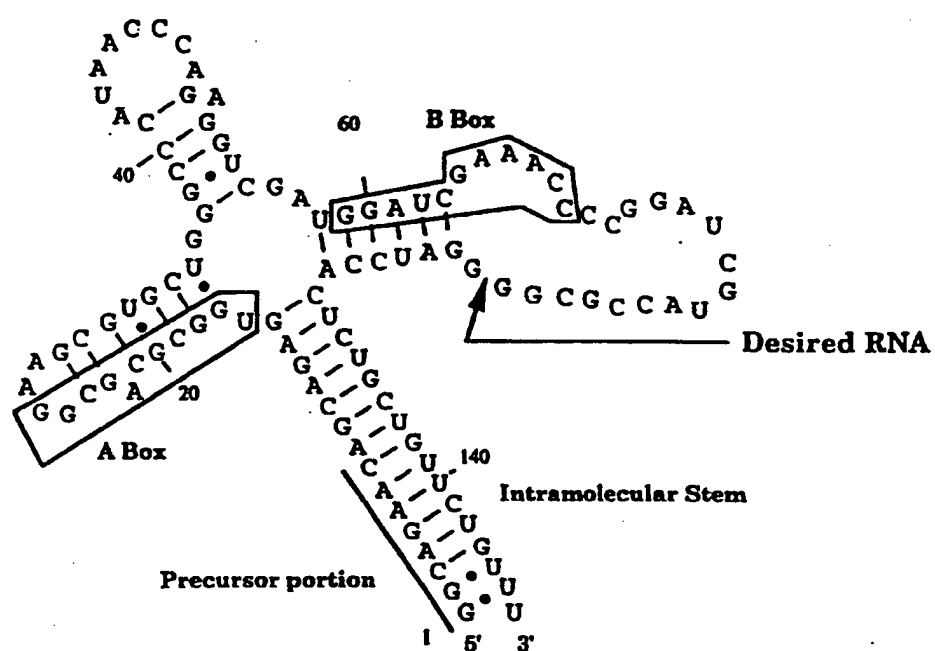


FIG. 40.

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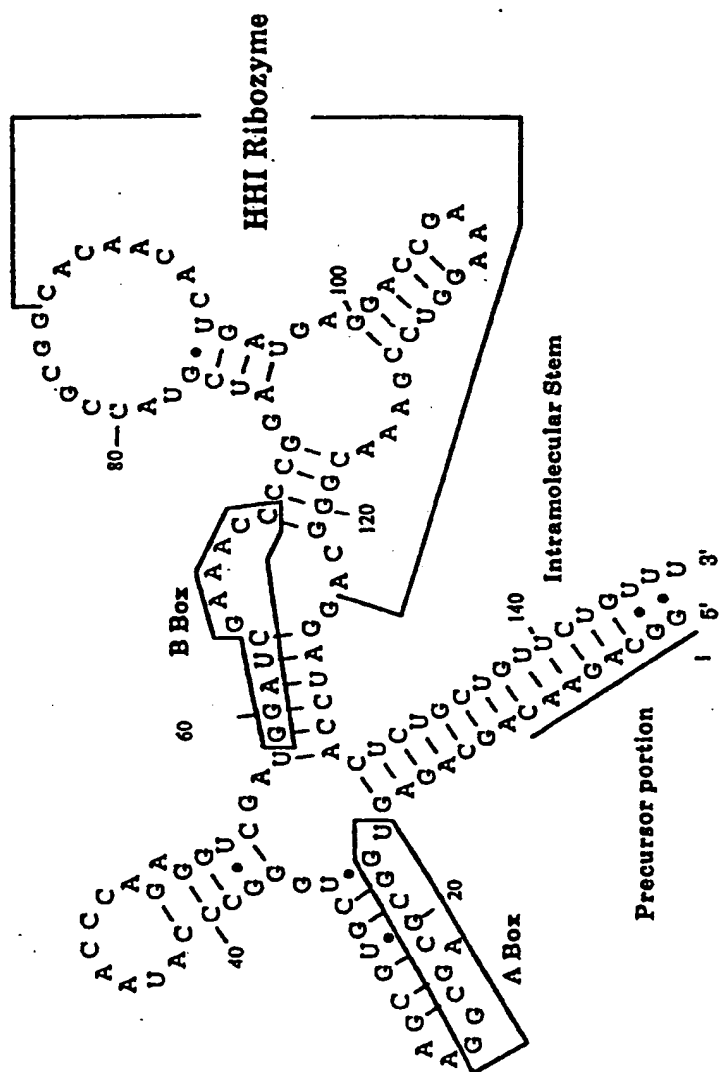
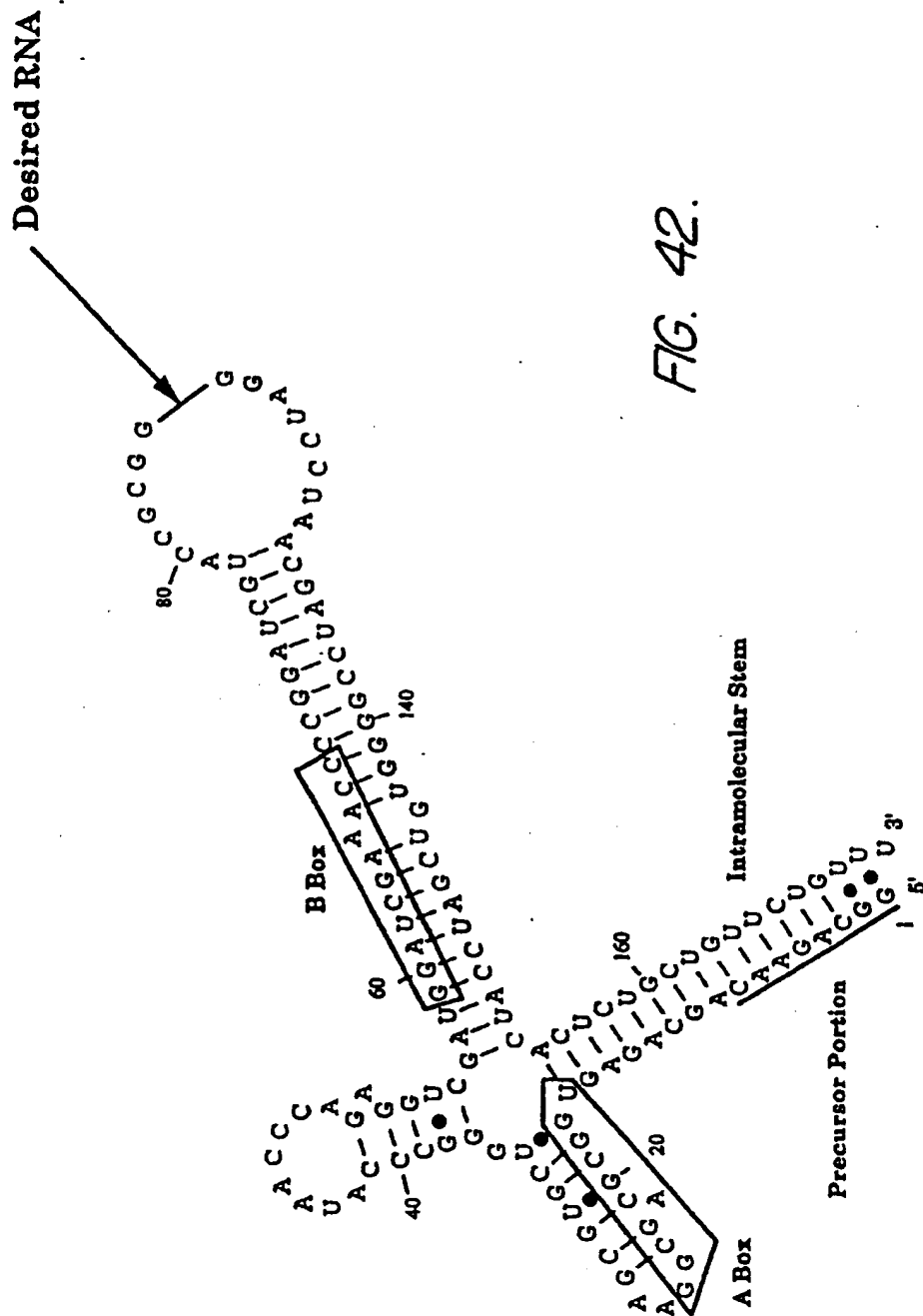


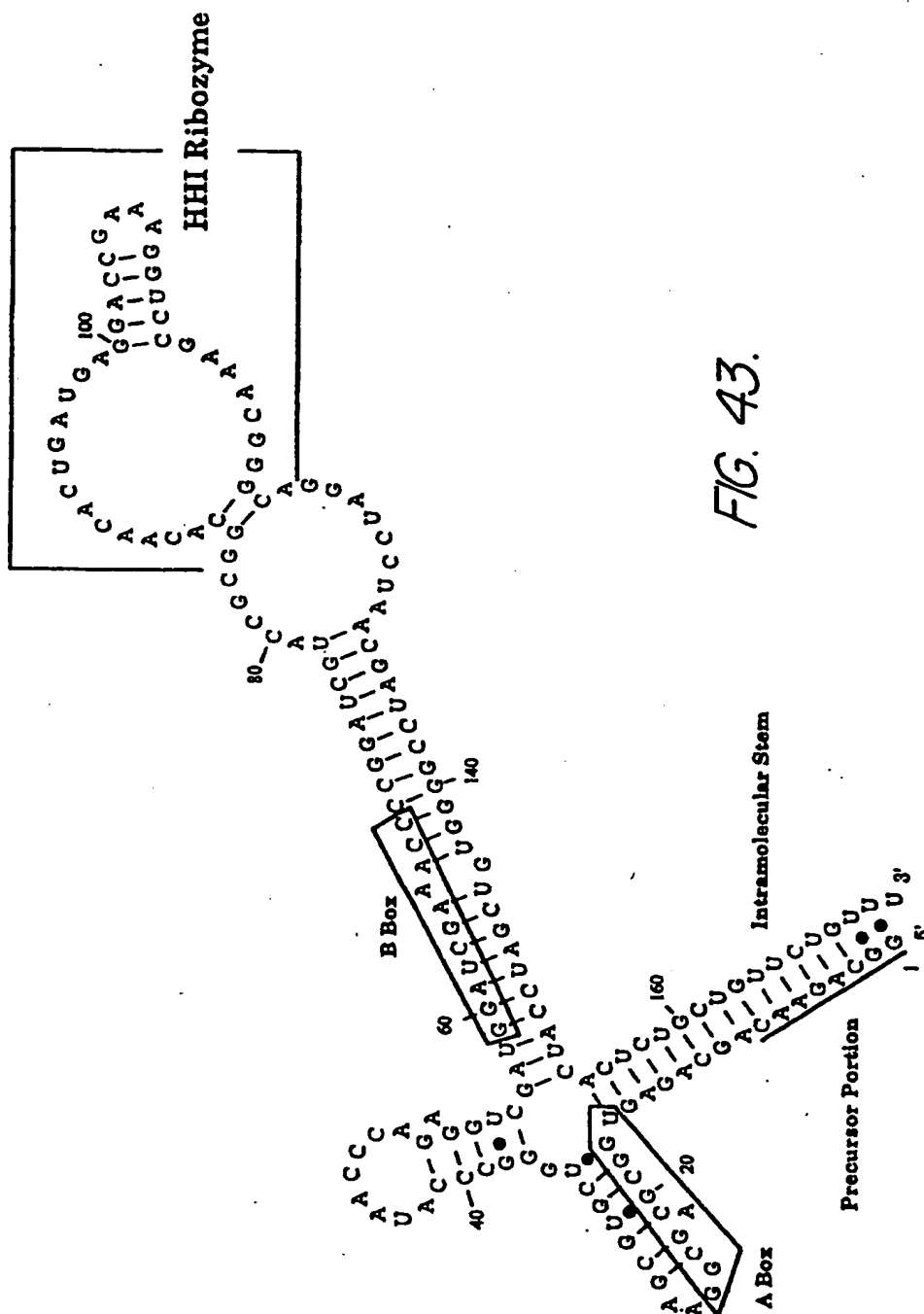
FIG. 41.

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S35 Sequence

FIG. 44.

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGUGGAU CCACUCUGCU 100
 GUUCUGUUU 109

FIG. 45.

HHIS35

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100
GACCGAAAGG UCCGAAACGG GCAGGAUCCA CUCUGCUGUU CUGUUU 146

Underlined bases indicate the HHI ribozyme sequence

FIG. 46.

S35 Plus Sequence

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGGGAUC CUAACGAUCC 100
 GGGGUGUCGA UCCAUCACUC UGCUGUUCUG UU U 133

FIG. 47.

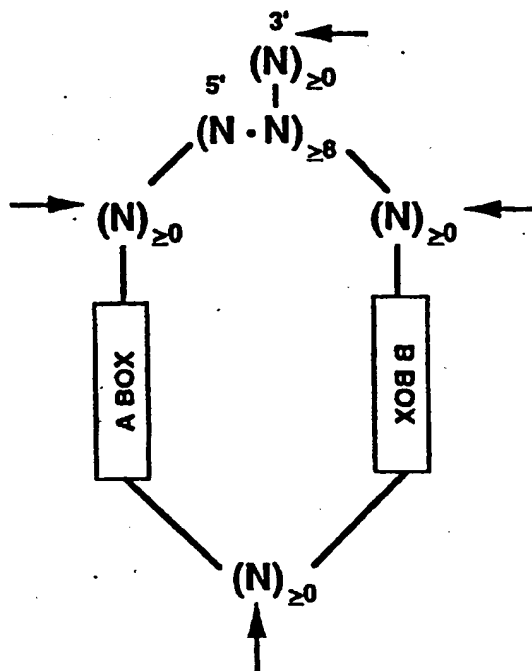
HHIS35 Plus

GGCAGAACAG CAGAGUGGCG CAGCGGAAGC GUGCUGGGCC CAUAACCCAG 50
 AGGUCGAUGG AUCGAAACCC CGGAUCGUAC CGCGGCACAA CACUGAUGAG 100
GACCGAAAGG UCCGAAACGG GCAGGAUCCU AACGAUCCGG GGUGUCGAUC 150
 CAUCACUCUG CUGUUCUGUU U 171

Underlined bases indicate the HHI ribozyme sequence
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FIG. 48.



A BOX = URGCNNAGYGG

B BOX = GGUUCGANUCC

This is based on Geiduschek & Tocchini-Valentini, (1988) *Annu. Review Biochem.* 57, 873-914. However this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

• = Indicates base-pairing

— = Indicates covalent linkage

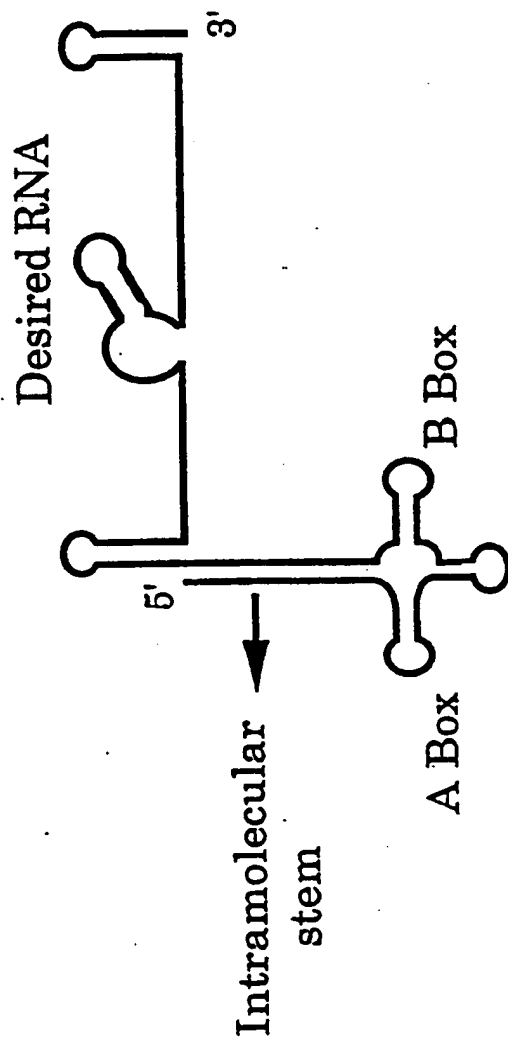
→ = Indicates sites at which desired RNAs can be cloned

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FIG. 49.



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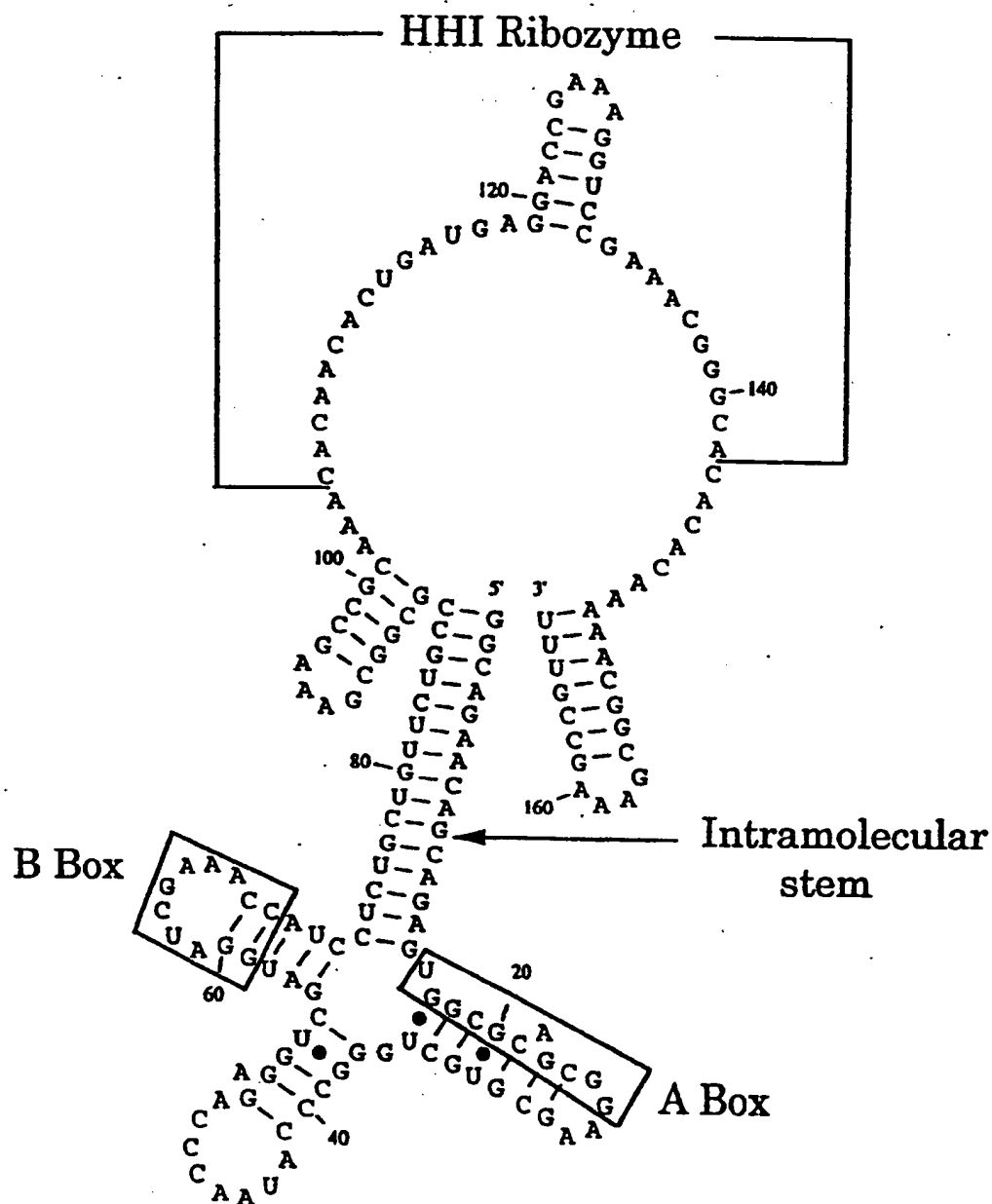


FIG. 51.

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FIG. 52a.

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A: TRZ-A

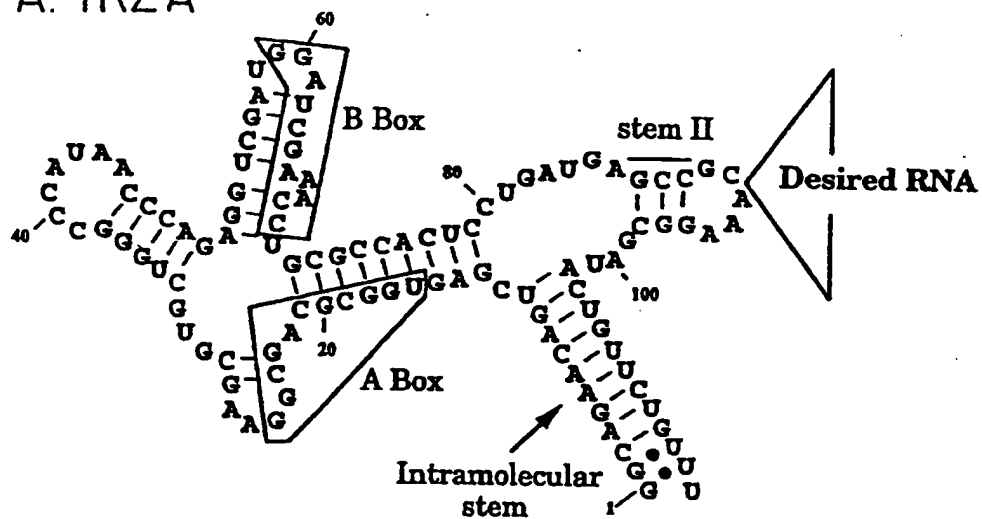
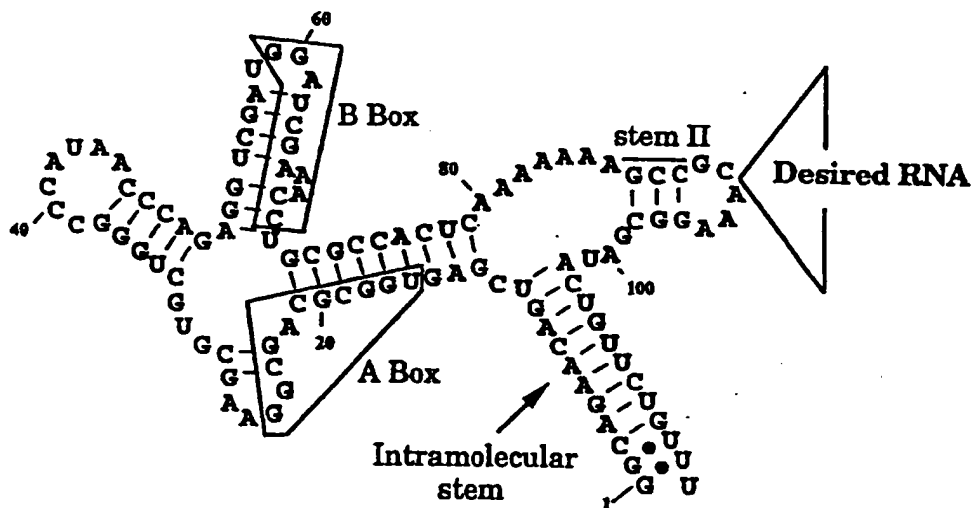


FIG. 52b.

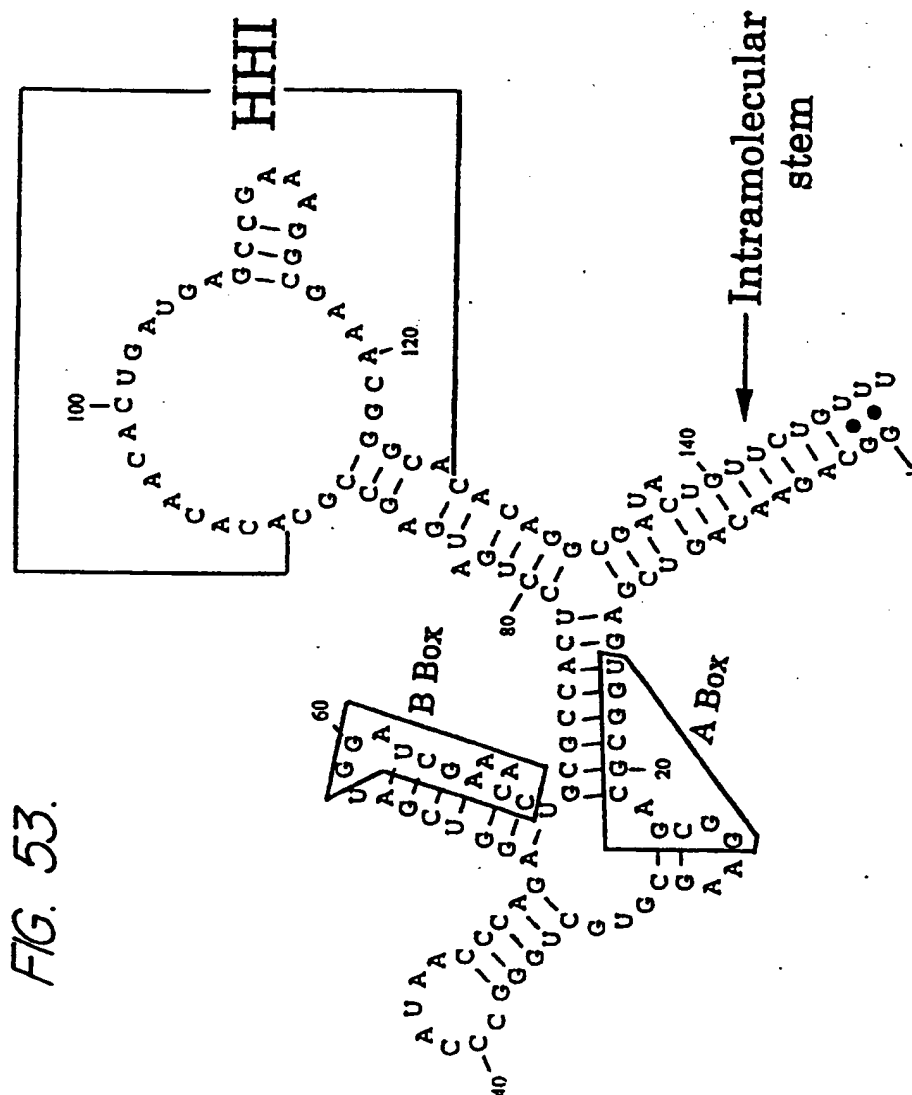
B: TRZ-B



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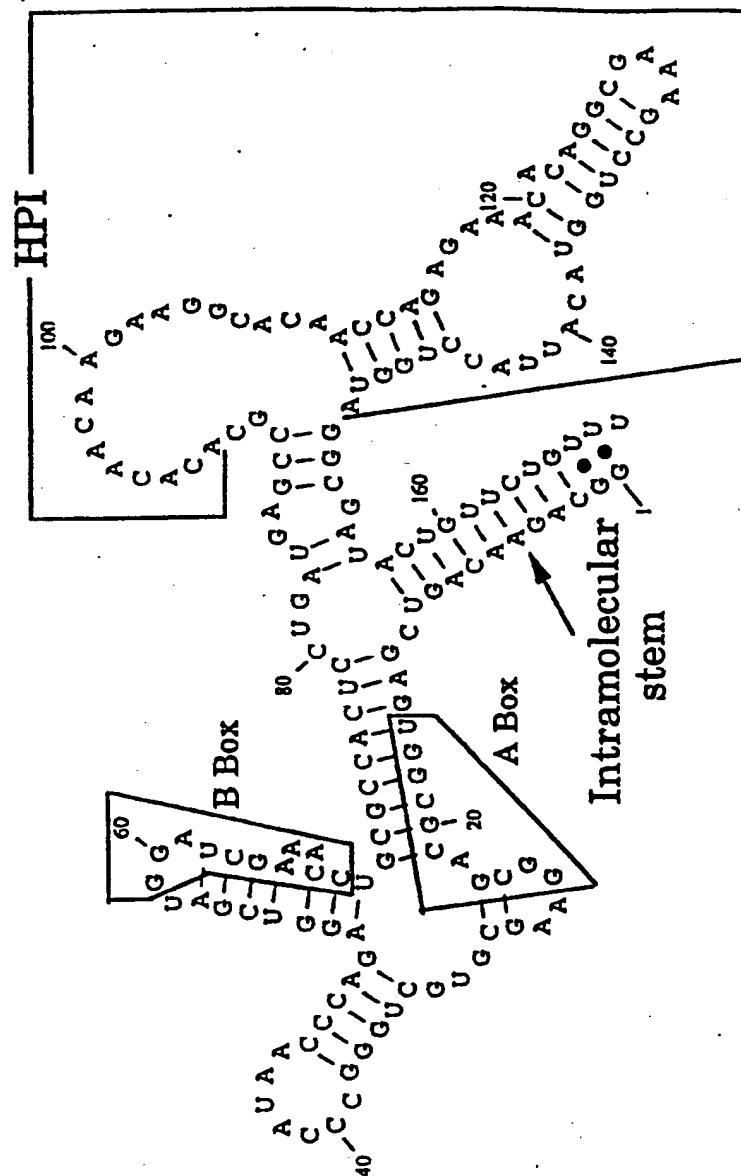


FIG. 54.

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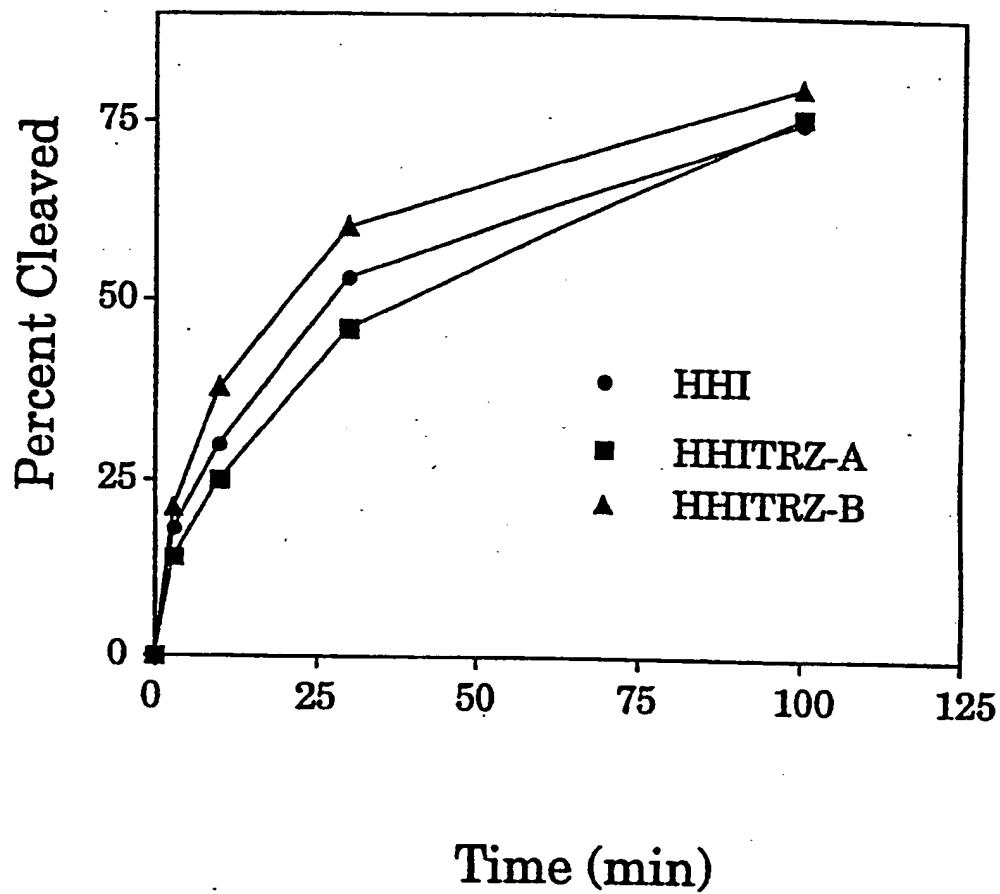


FIG. 55.

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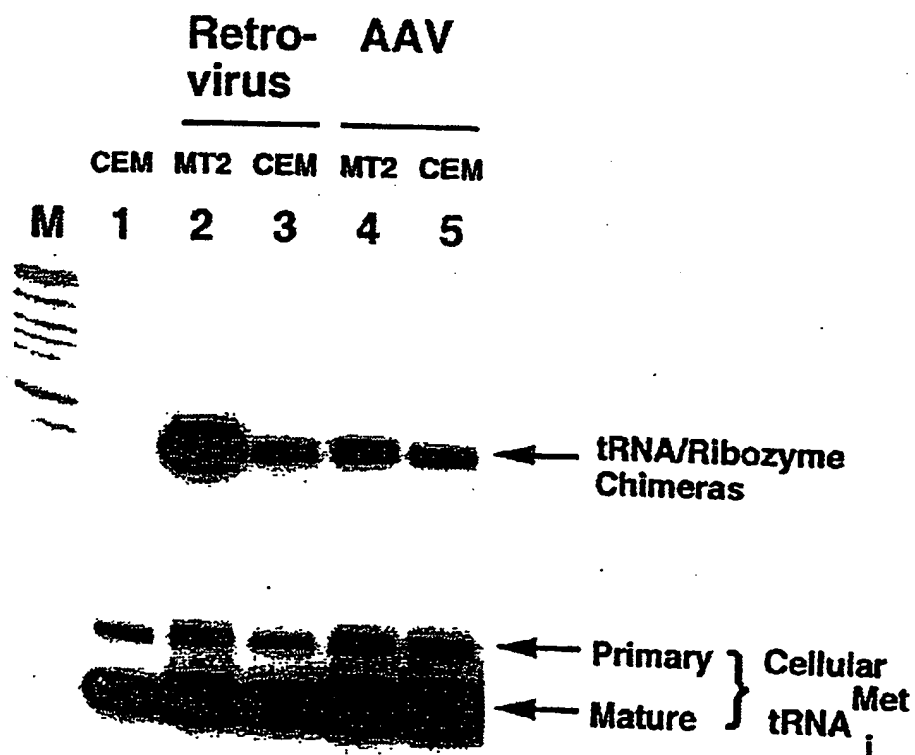


FIG. 56.

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FIG. 57a.

AAV Vector

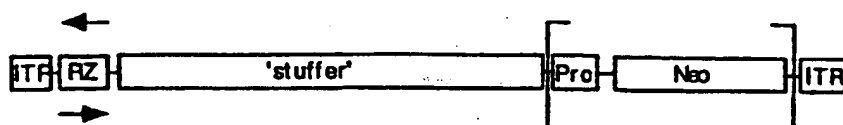
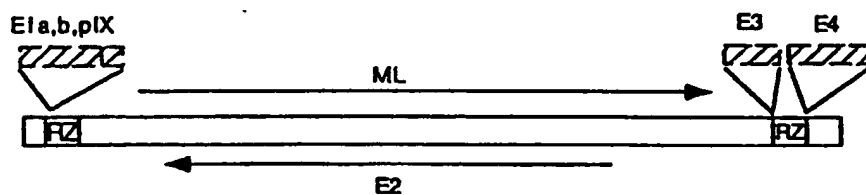


FIG. 57b.

Adenovirus Vector



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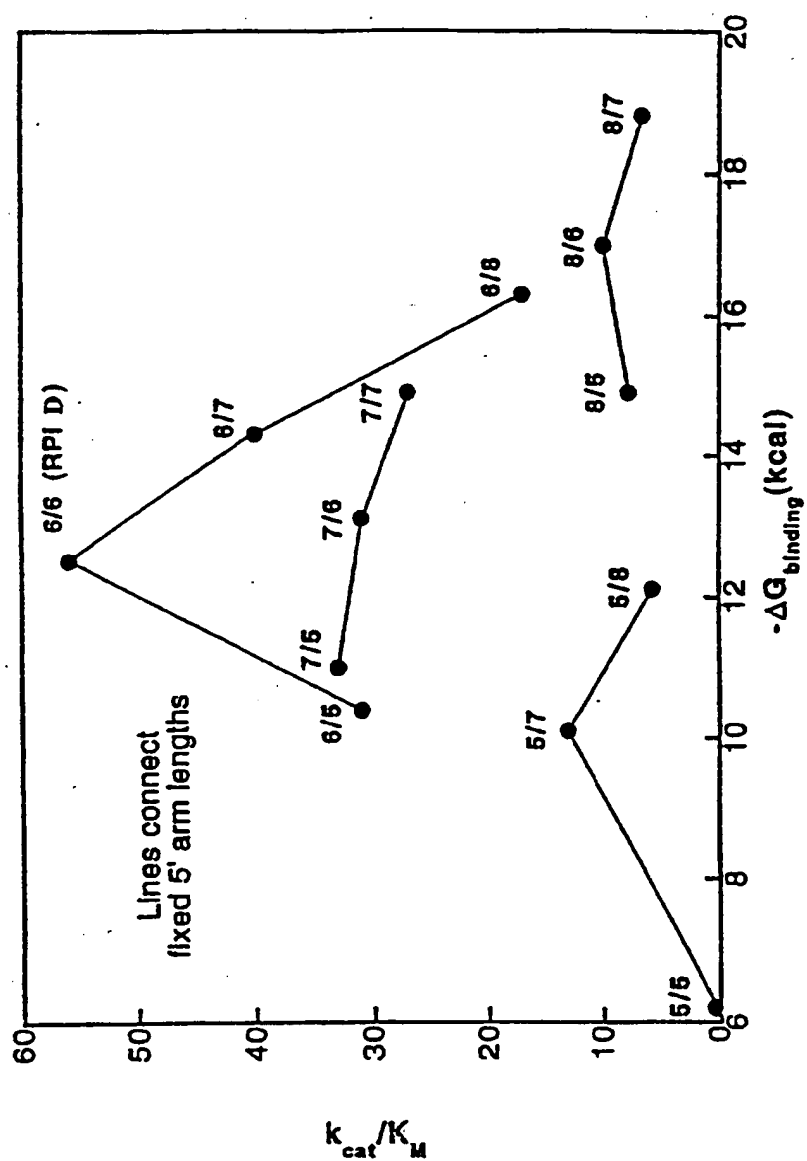
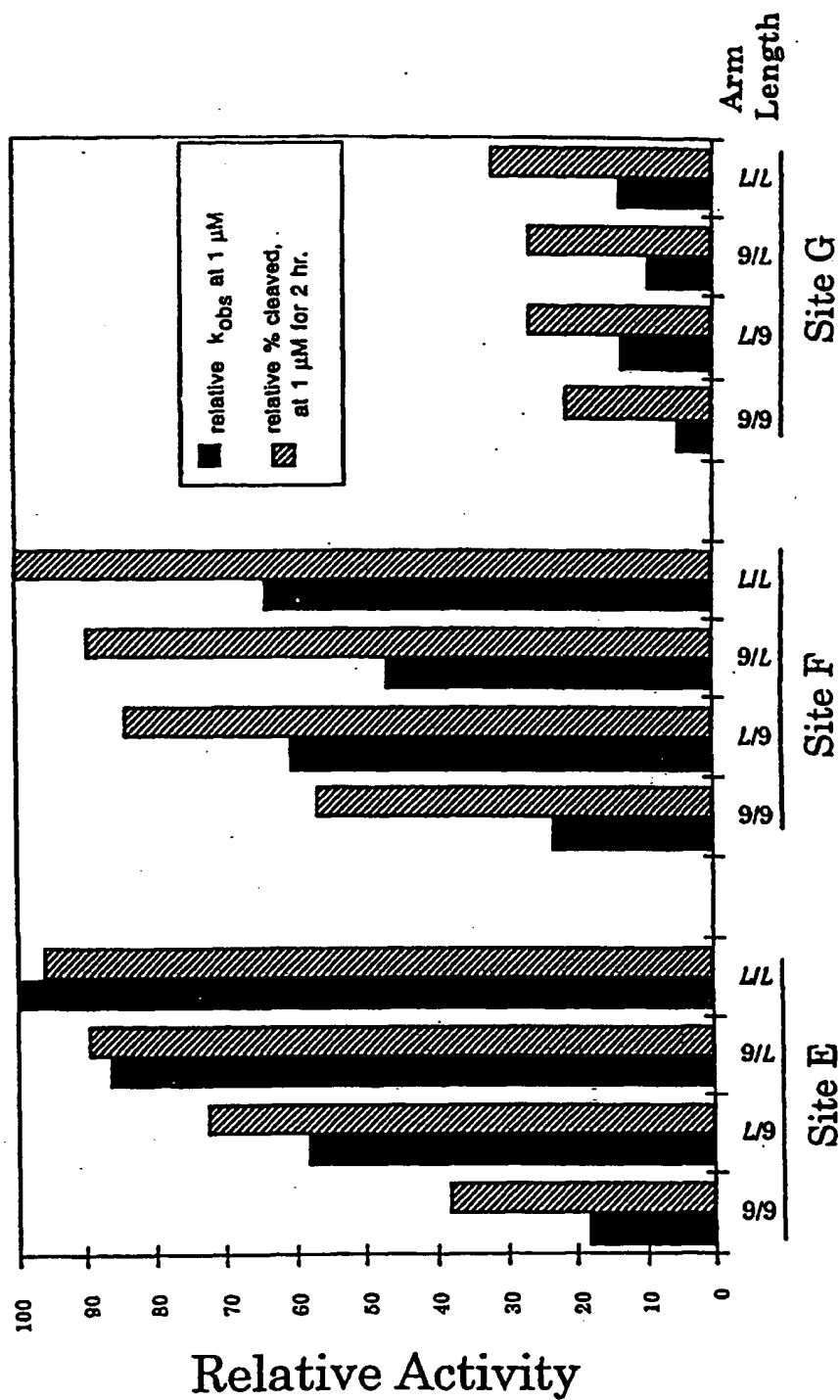


FIG. 58.

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Ribozyme

FIG. 59.

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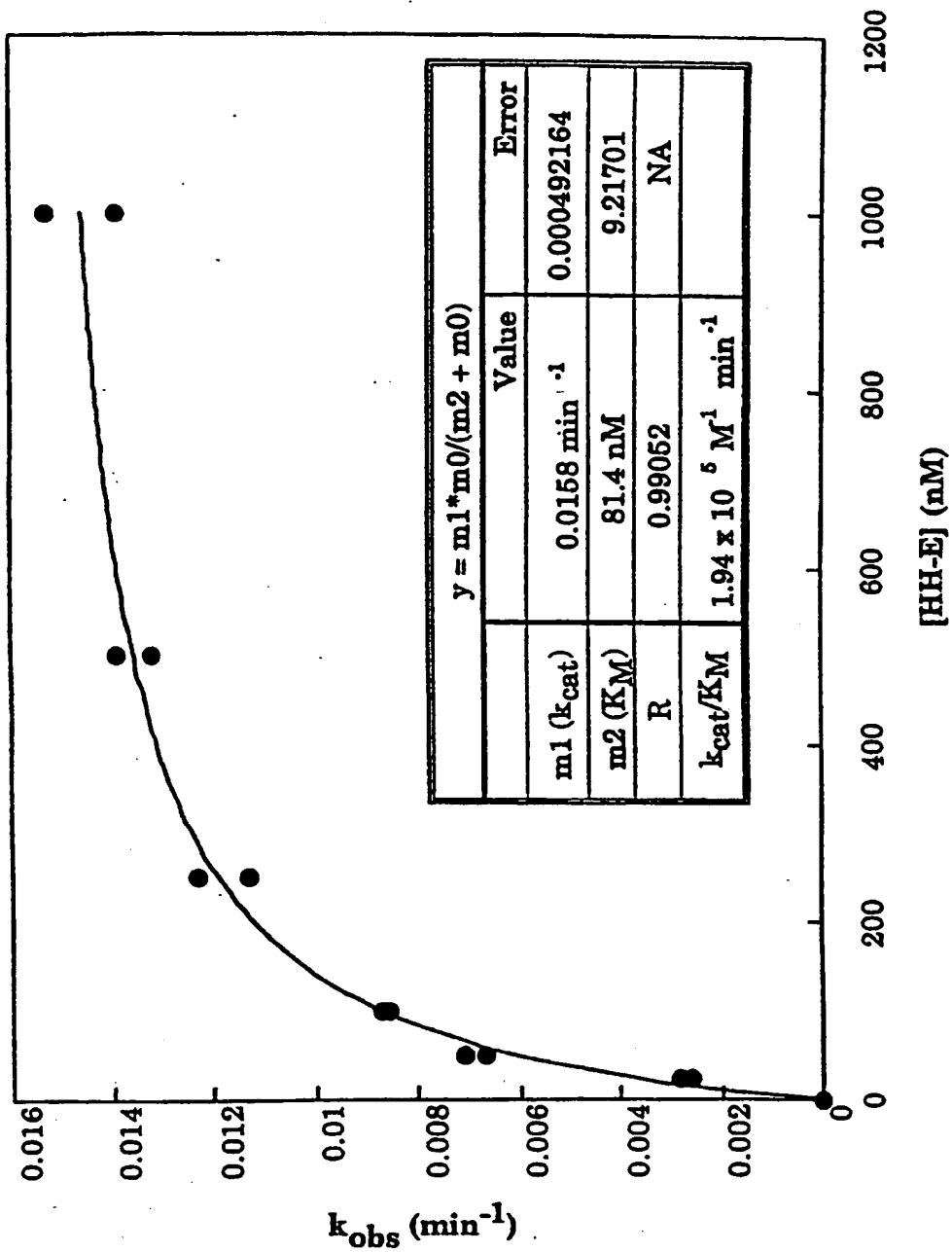
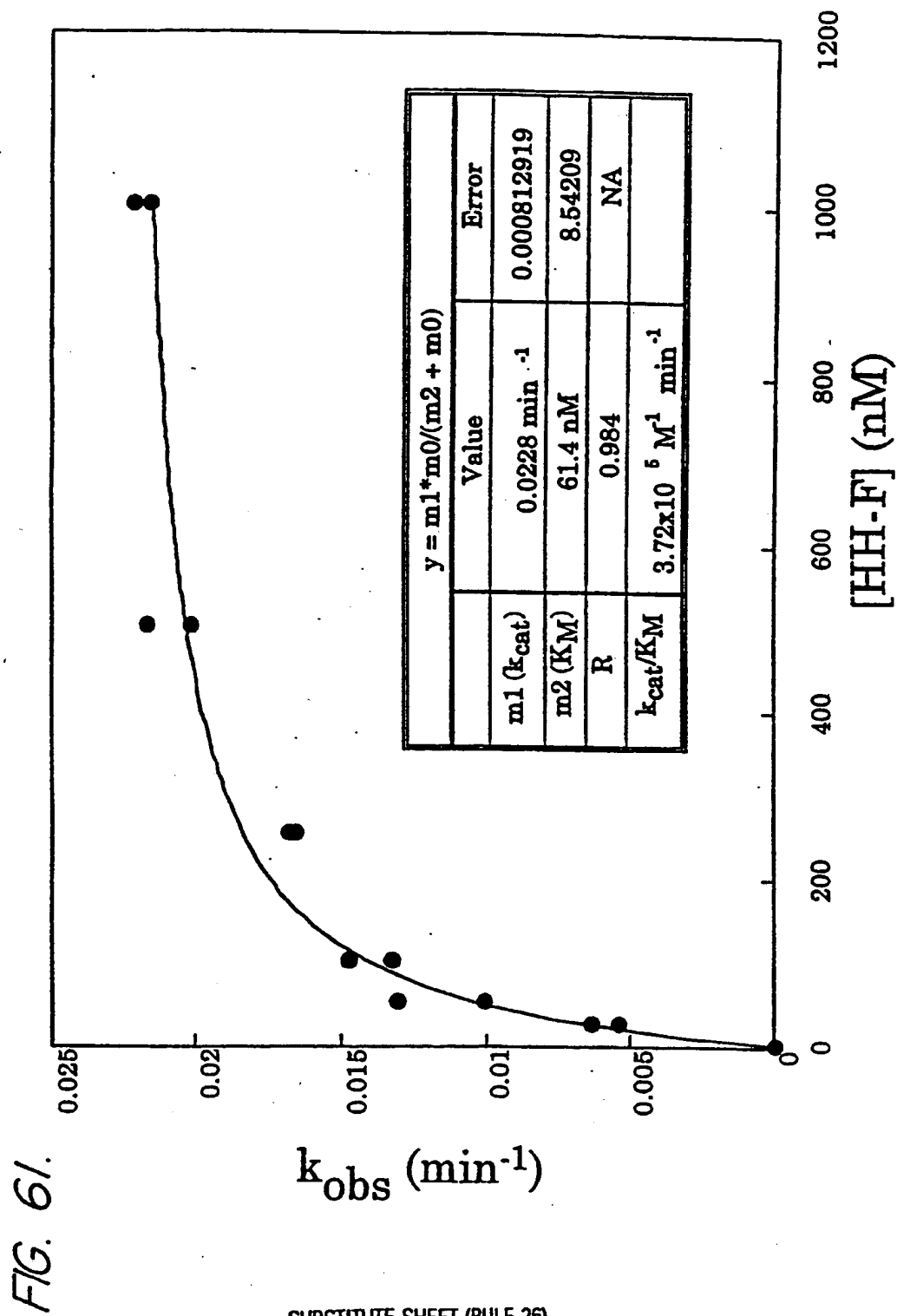


FIG. 60.

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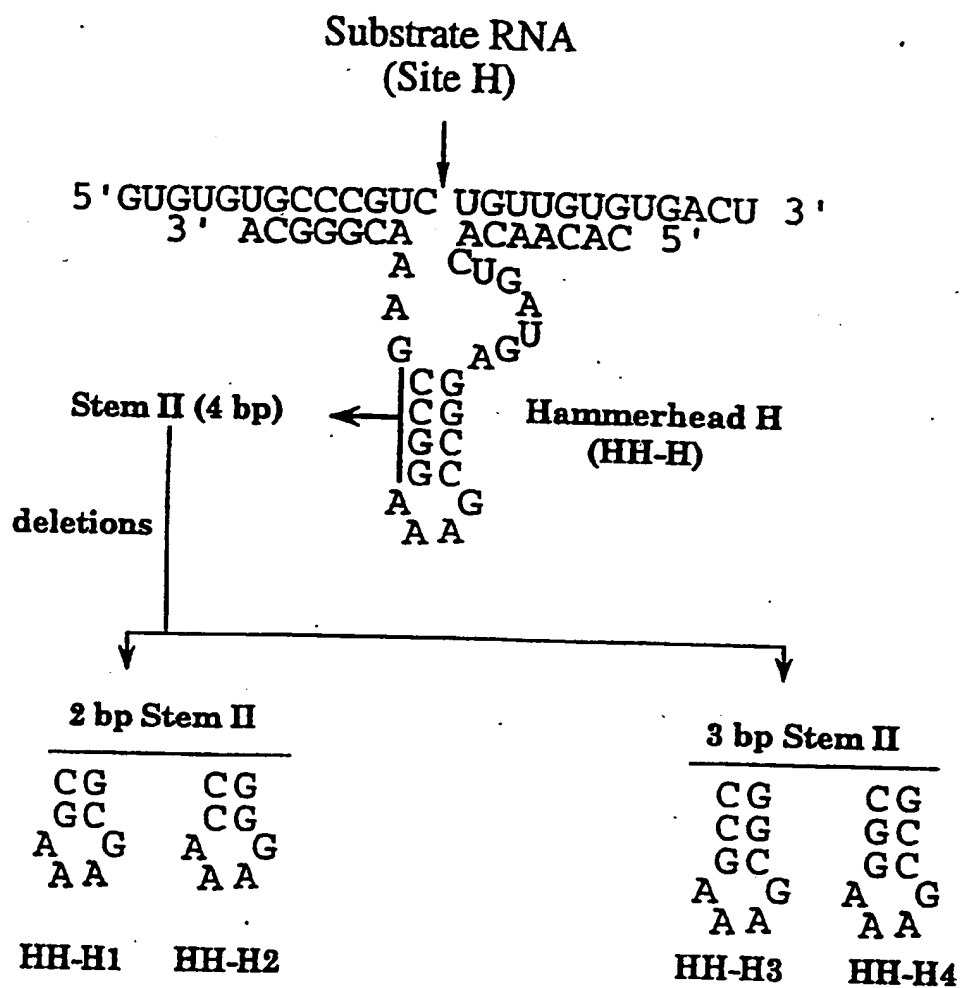


FIG. 62.

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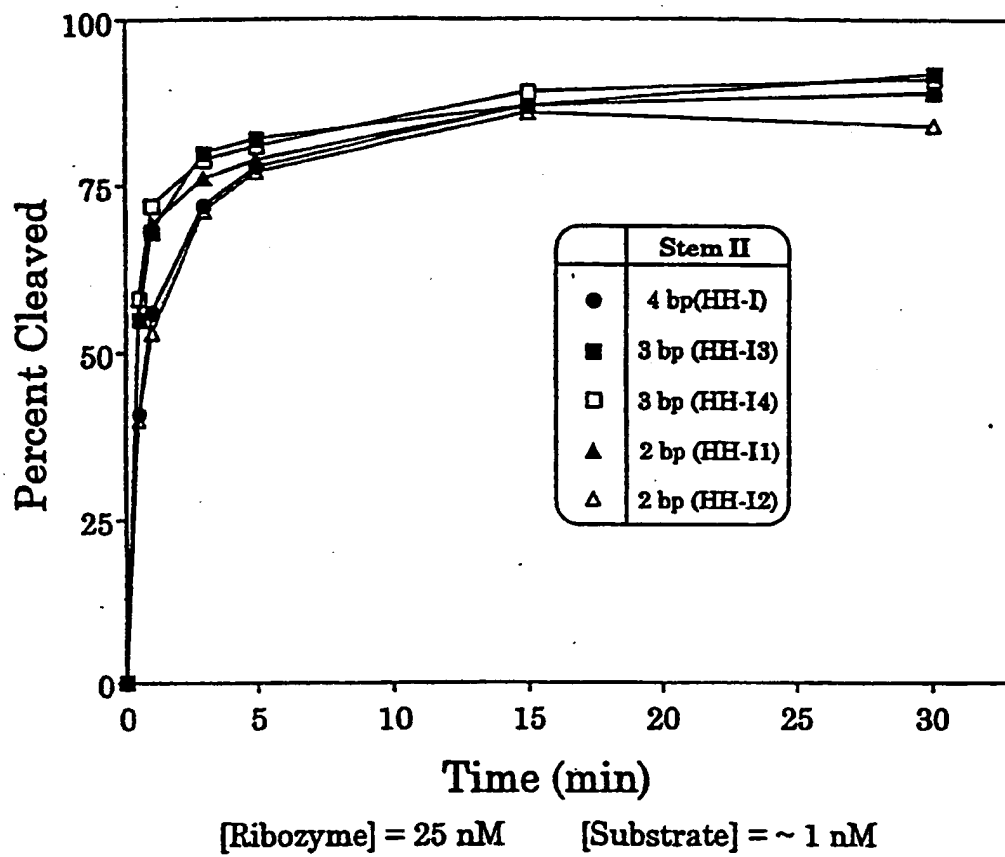
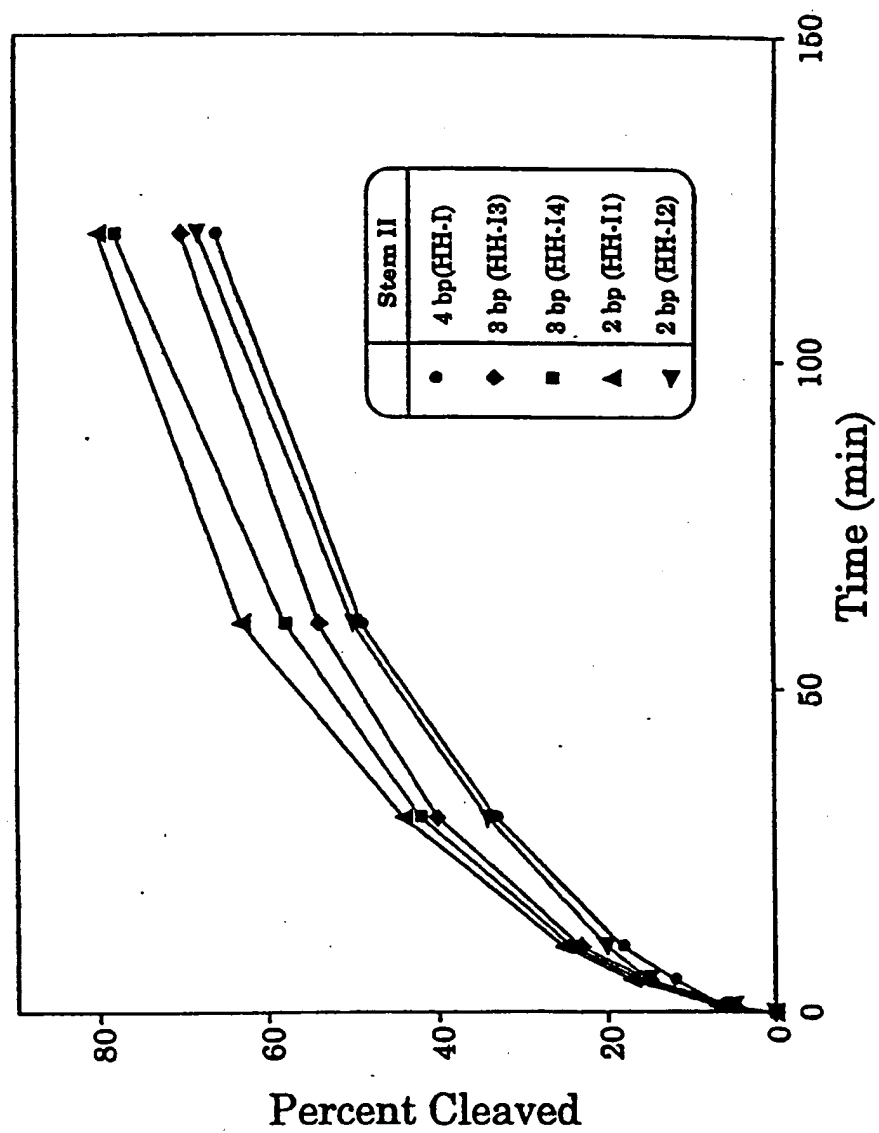


FIG. 63.

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[Ribozyme] = 1000 nM [Long Substrate] = ~10 nM

FIG. 64.

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FIG. 65a.

Substrate RNA (site J)

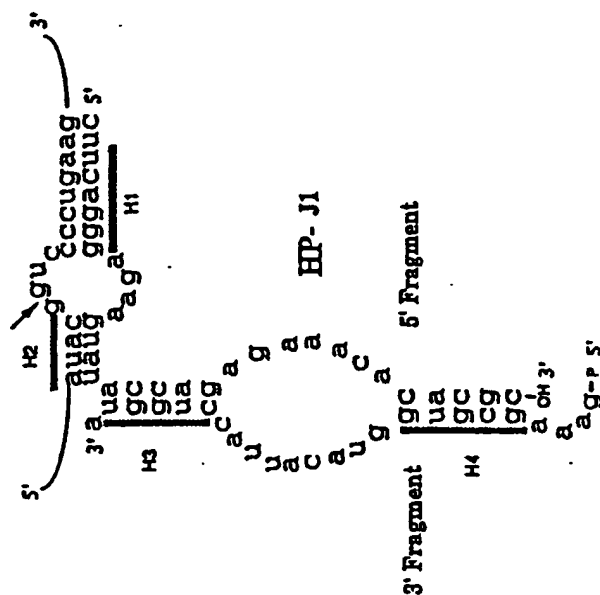
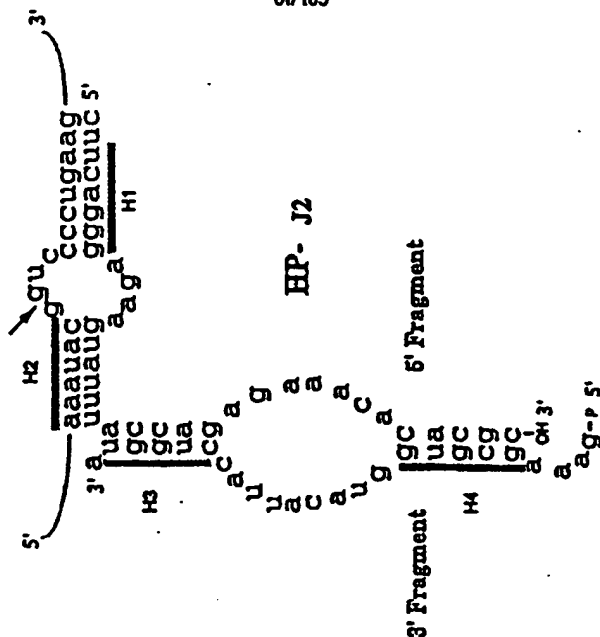


FIG. 65b.

Substrate RNA (site J)



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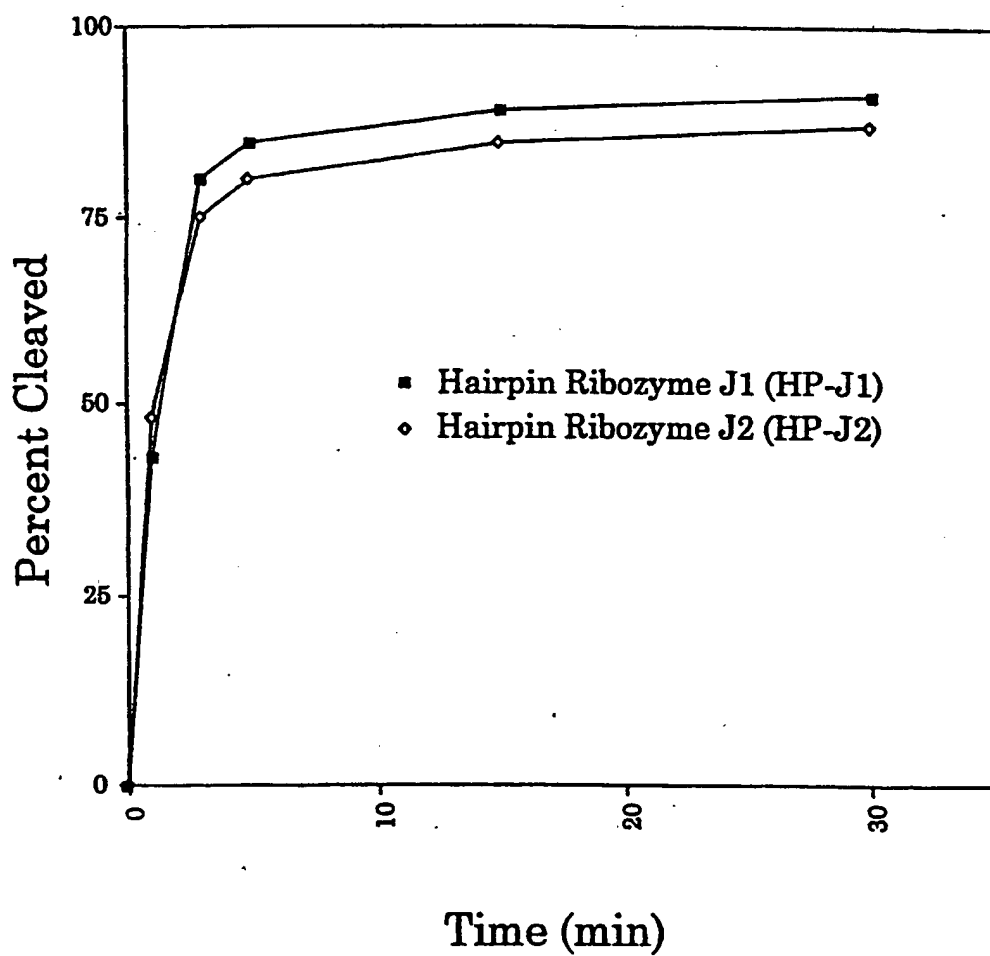


FIG. 66.

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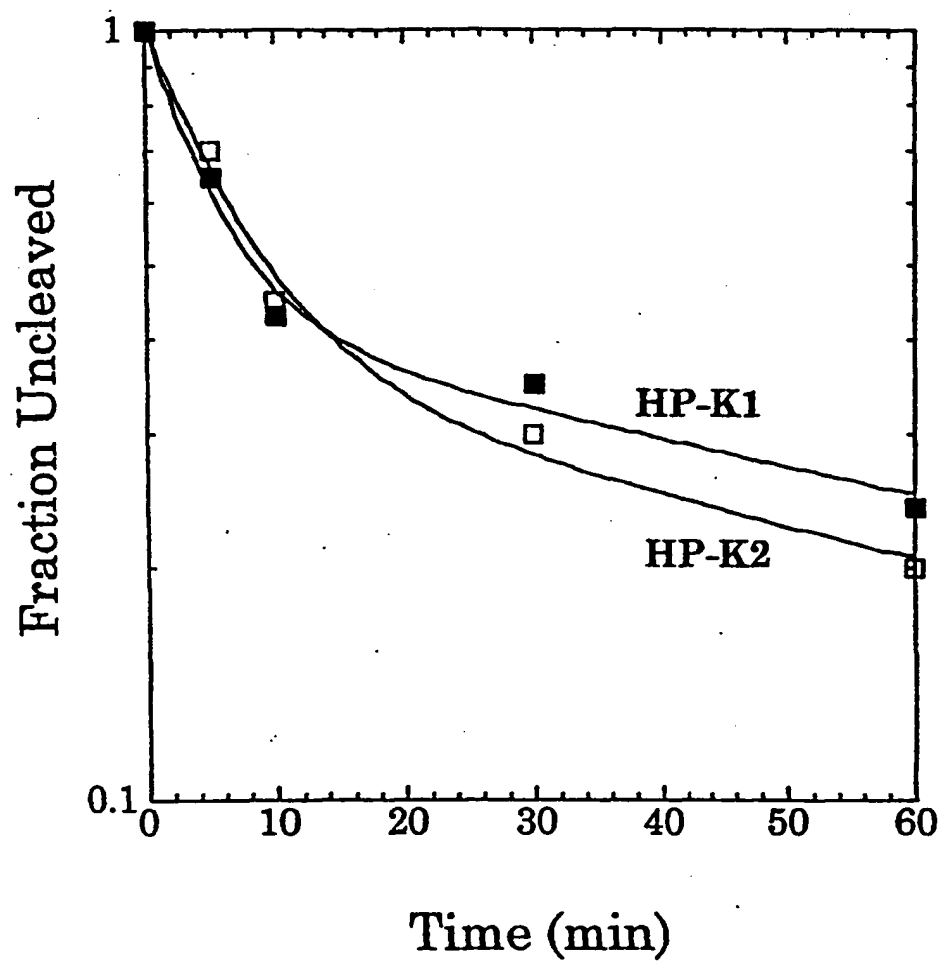


FIG. 68.

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FIG. 69b.

Substrate RNA

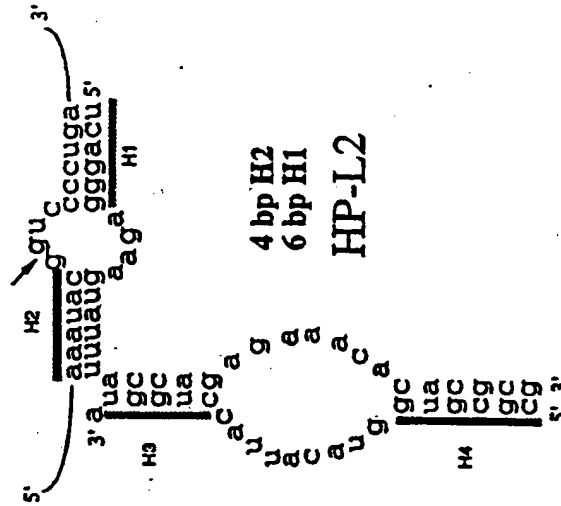
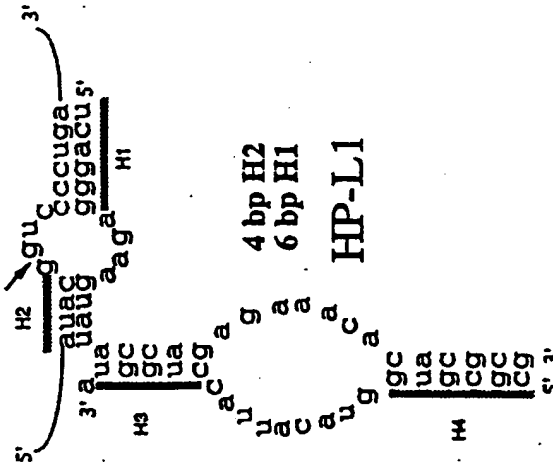


FIG. 69a.

Substrate RNA



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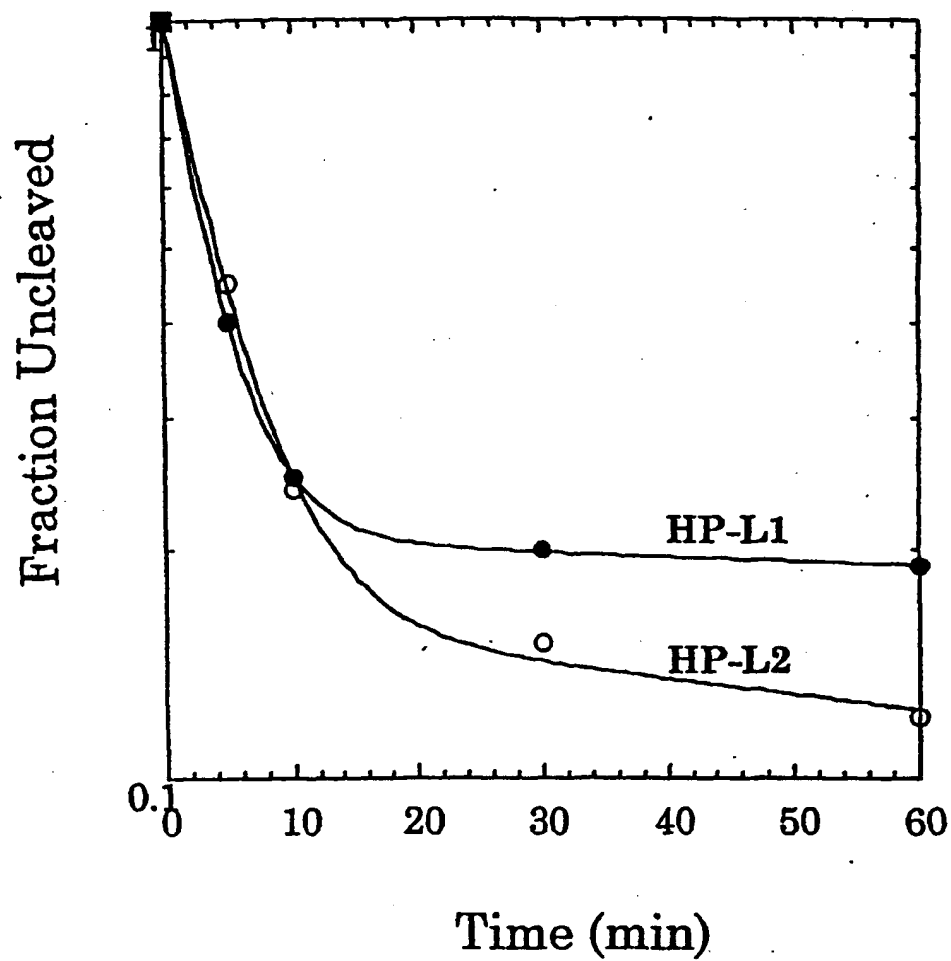


FIG. 70.

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FIG. 71b.

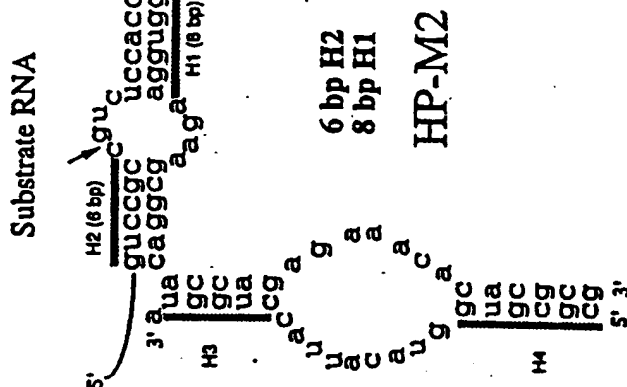
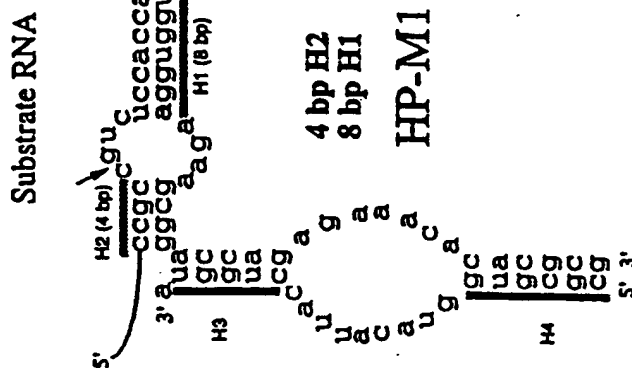


FIG. 71a.



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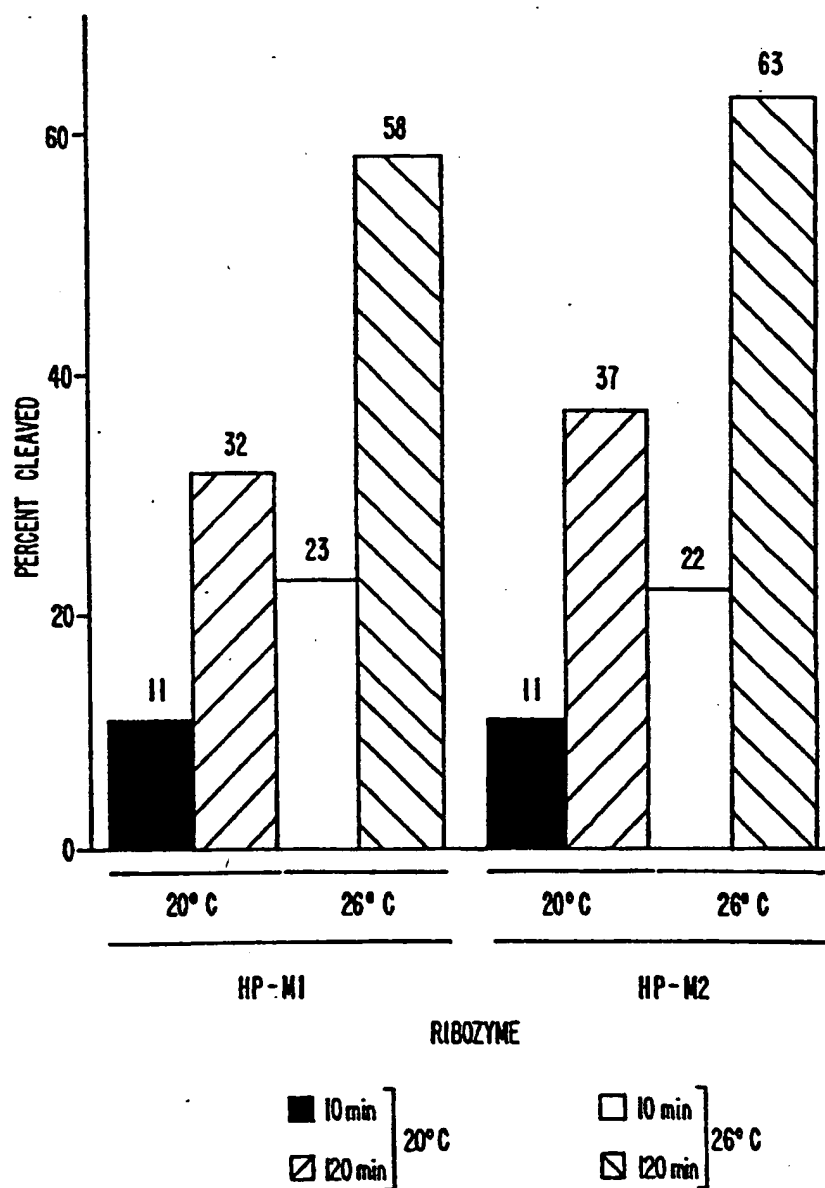
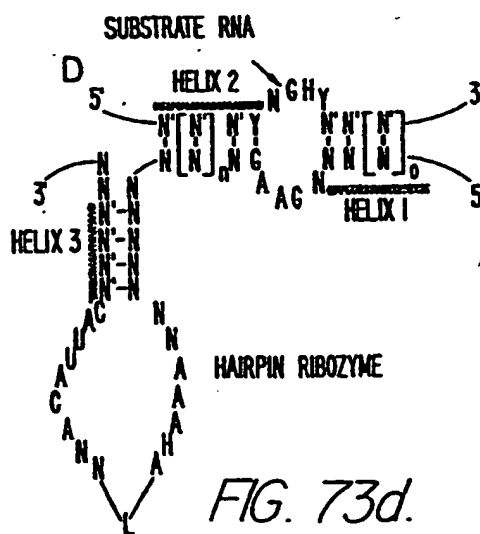
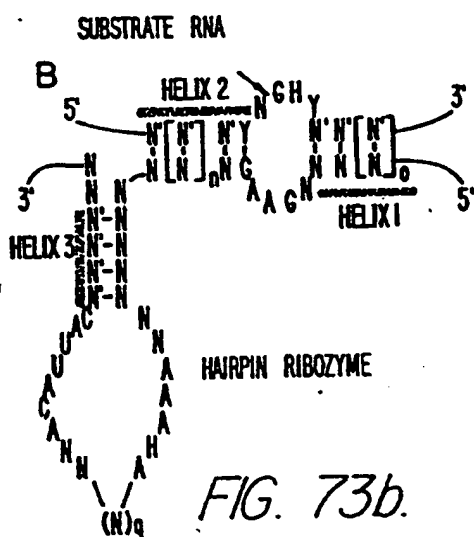
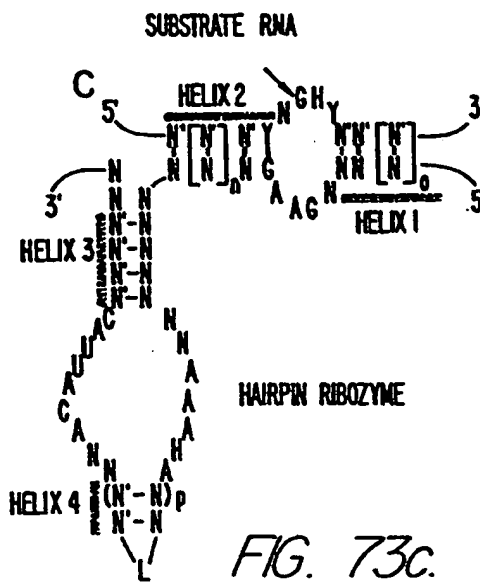
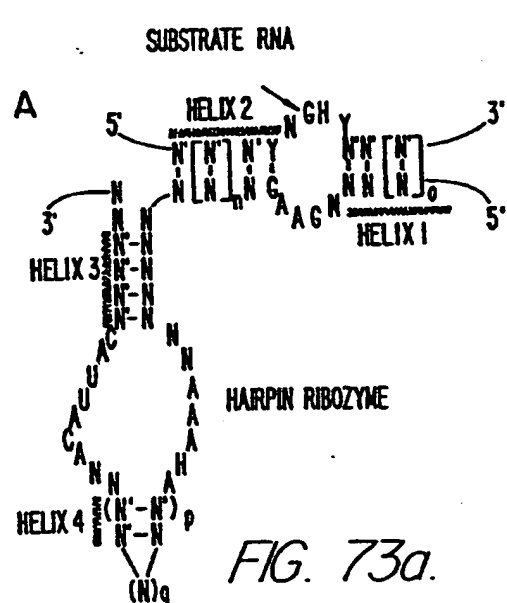


FIG. 72.

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FIG. 74b.

B

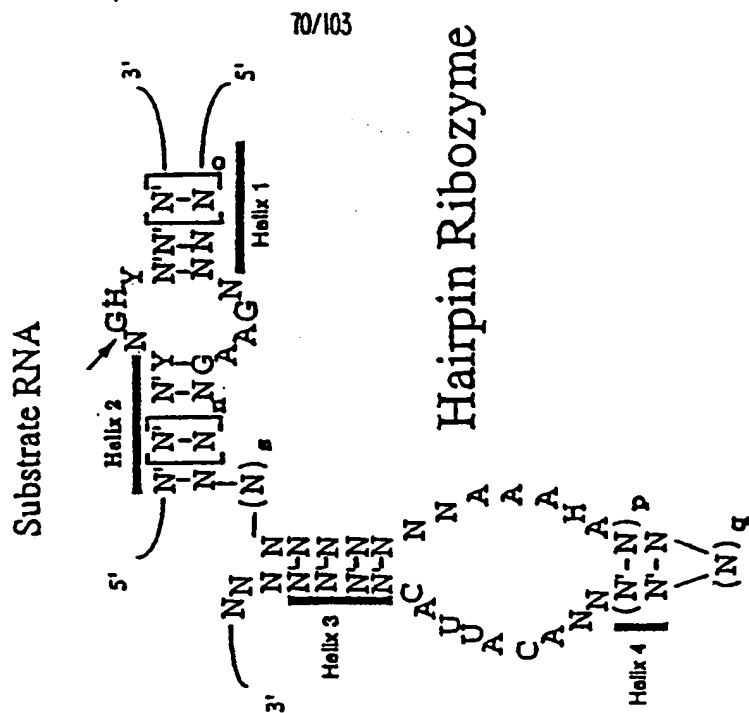
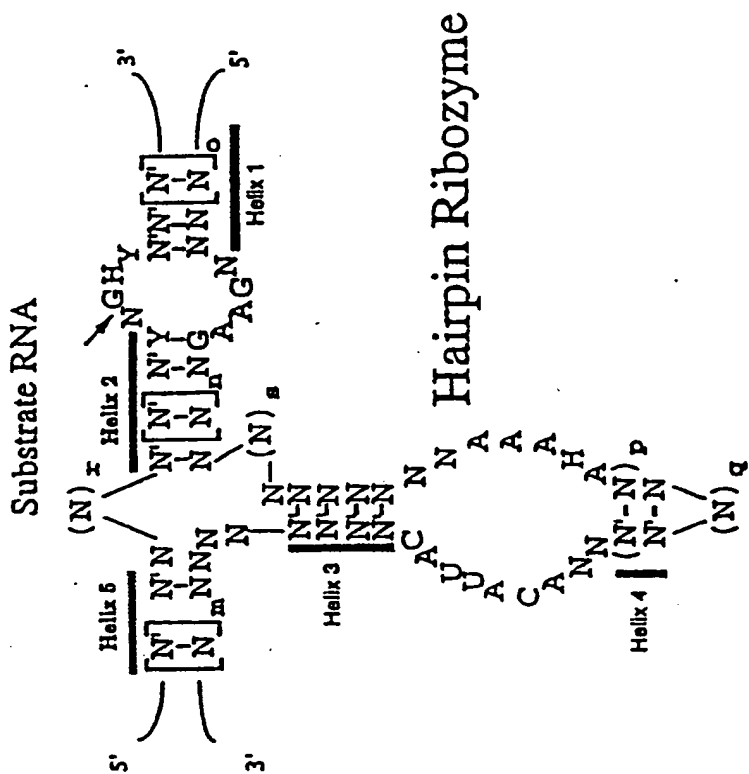


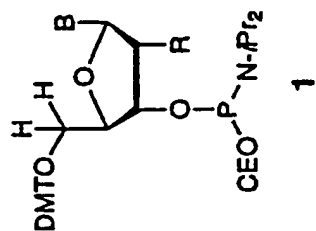
FIG. 74a.

A



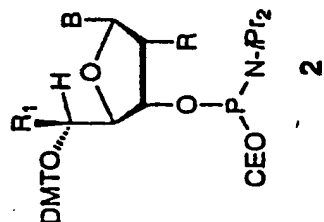
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FIG. 75a.



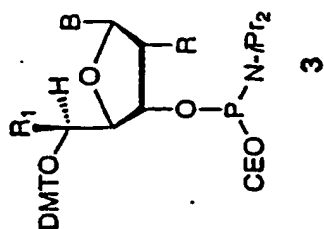
D-Ribose Family

FIG. 75b.



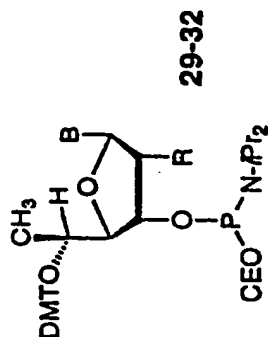
D-Allose Family

FIG. 75c.



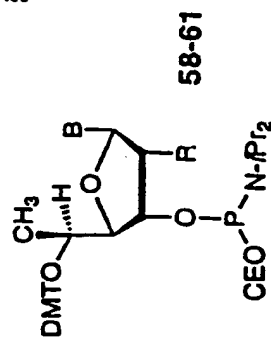
L-Talose Family

FIG. 75d.



D-Allose

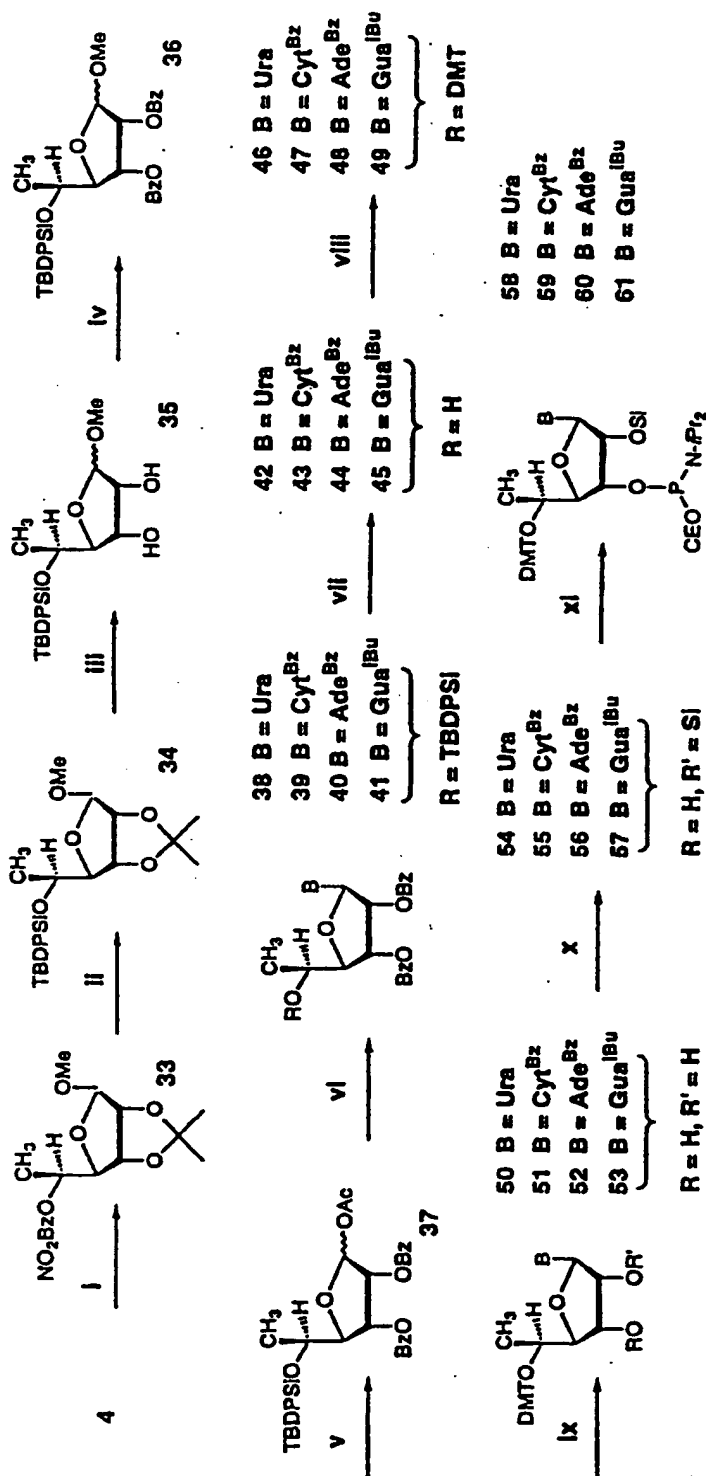
FIG. 75e.



L-Talose

B = Protected A, C, G, U, T, 2AP, I, DIAP, P etc.

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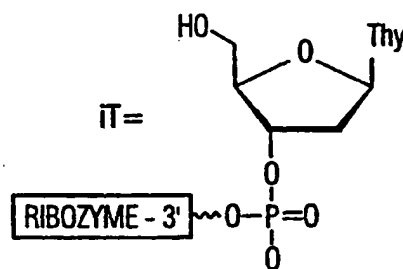
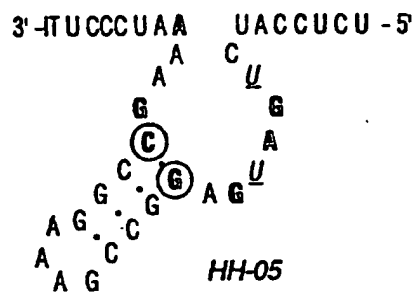
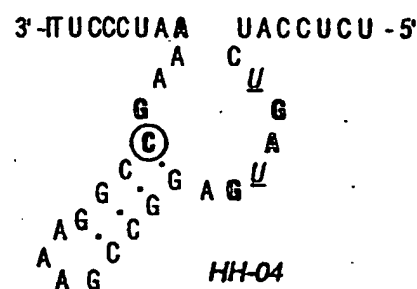
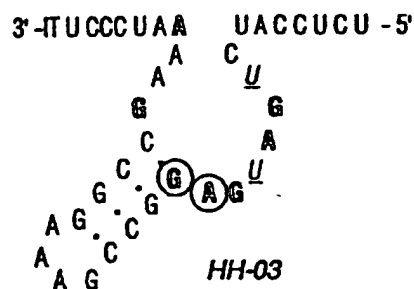
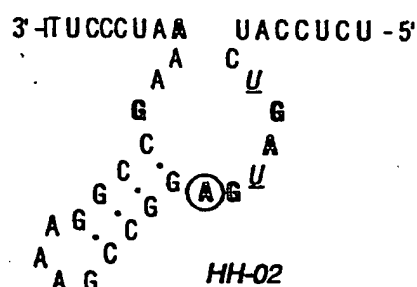
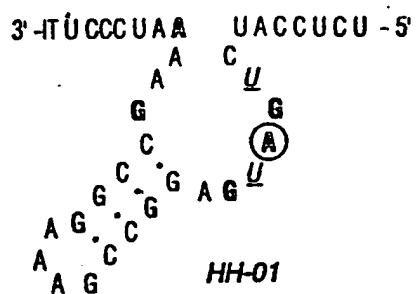
- Legend for R groups:
- I) = Ph₃P/DEAD/p-NO₂PhCOOH
 - II) = OH⁺, TBDPSI-Cl
 - III) = H⁺
 - IV) = Bz-Cl/Pyr
 - V) = AcOH/Ac₂O/H⁺
 - VI) = TMS/CF₃SO₃SiMe₃
 - VII) = TBAF
 - VIII) = DMT-Cl/AgNO₃
- Legend for X groups:
- IX) = OH⁺
 - X) = TBDMSI-Cl
 - XI) = P(OCE)(N-IPr₂)Cl

FIG. 77.

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FIG. 78.



N=2'-O-Me	Ⓜ=RIBO
U=2'-NH ₂ U	Ⓢ=TALO

WHERE THE ALPHABET "N" REPRESENTS A NUCLEOTIDE, A, U, G, OR C
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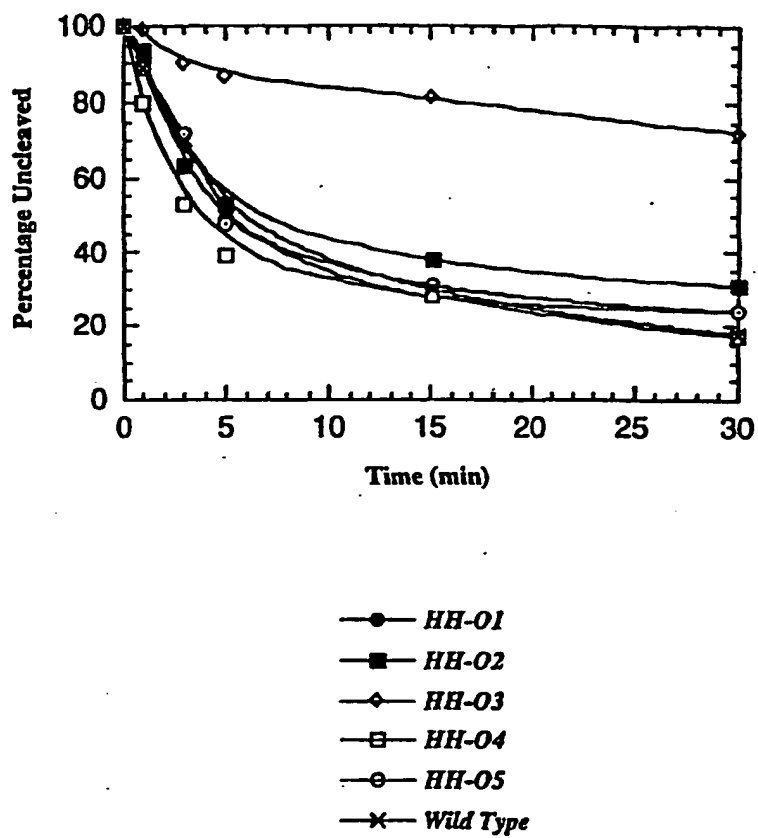


FIG. 79.

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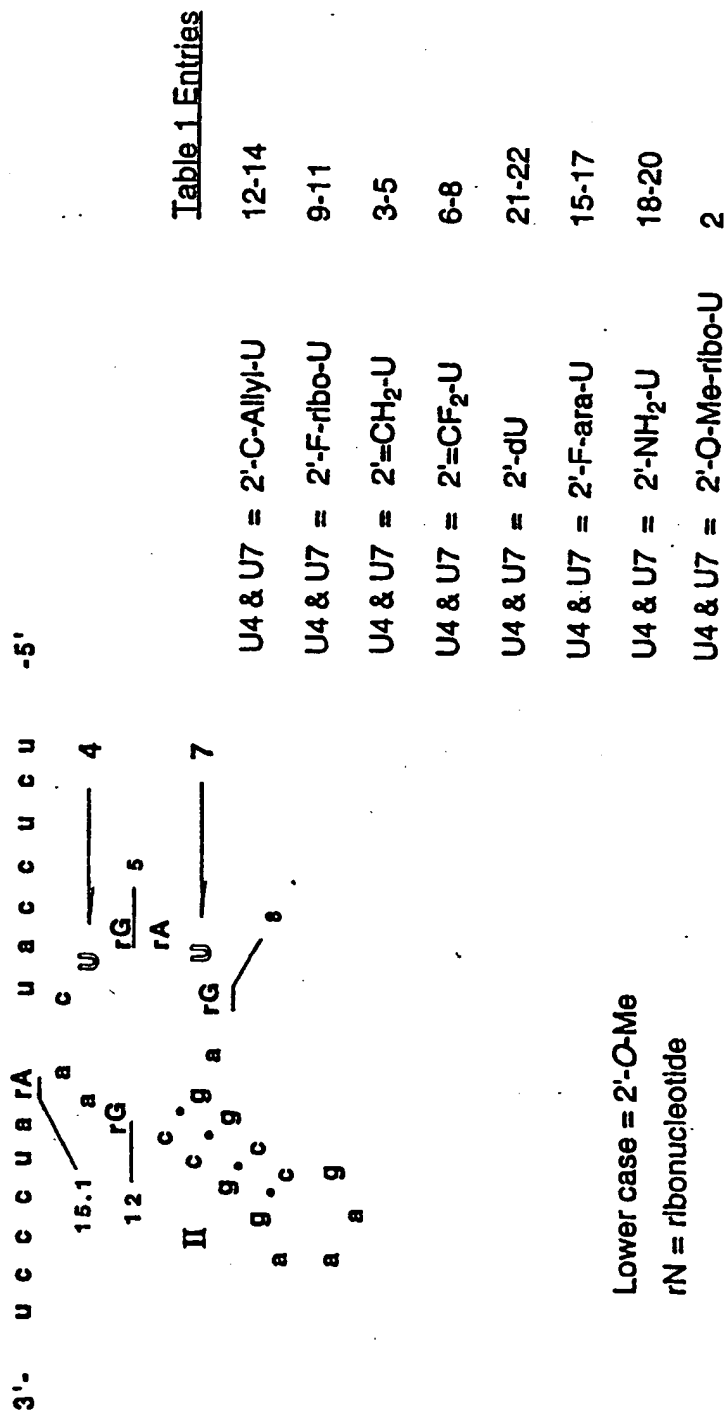


FIG. 80.

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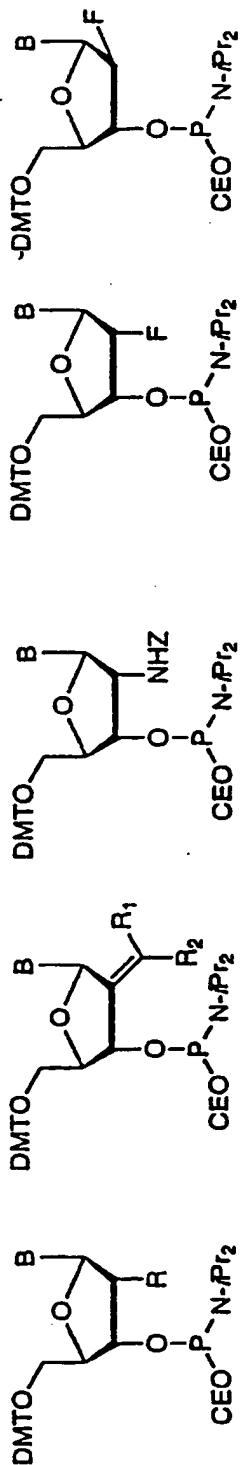


FIG. 8Ia.

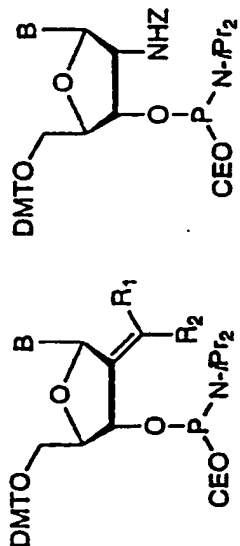


FIG. 8Ib.

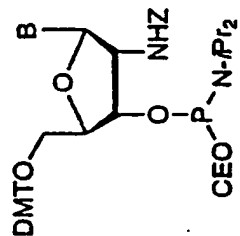


FIG. 8Ic.

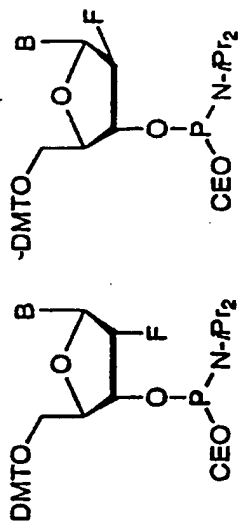


FIG. 8Id.

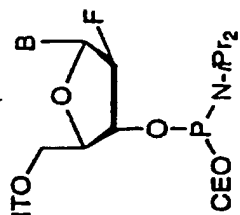


FIG. 8Ie.

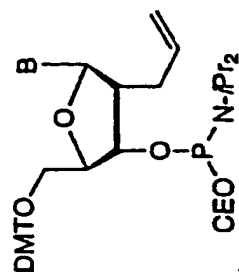


FIG. 8If.

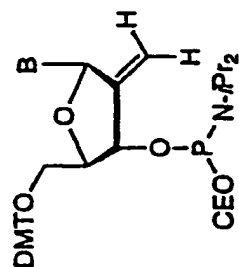


FIG. 8Ig.

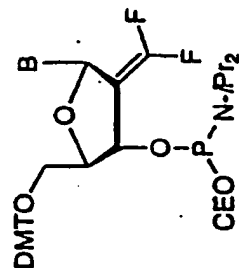


FIG. 8Ih.

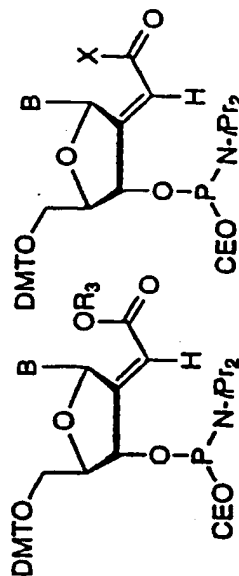


FIG. 8Ii.

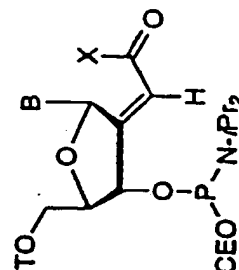


FIG. 8Ij.

SUBSTITUTE SHEET (RULE 26)

B = Protected A, C, G, U, T, 2AP, I, DIAP, P etc.

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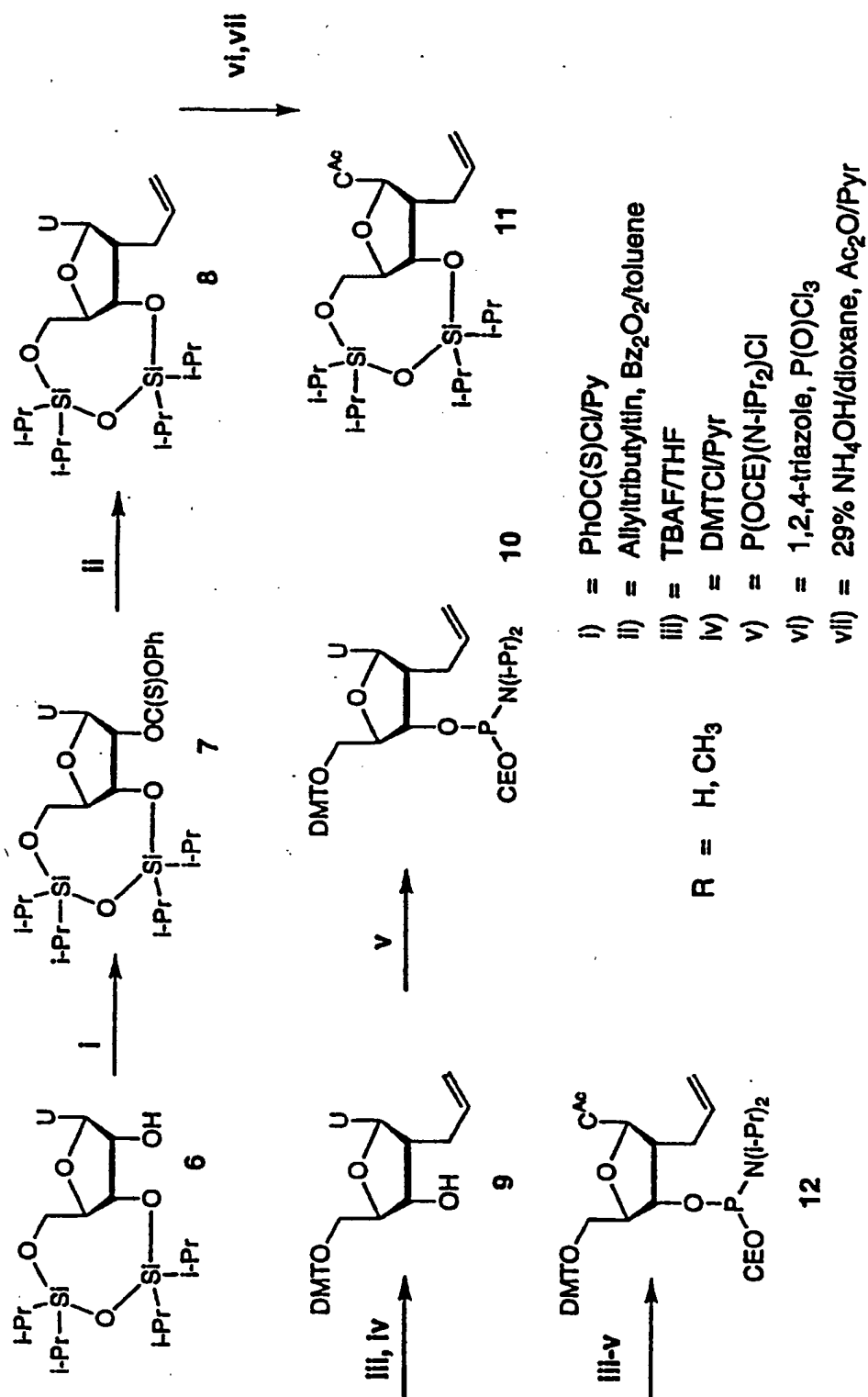
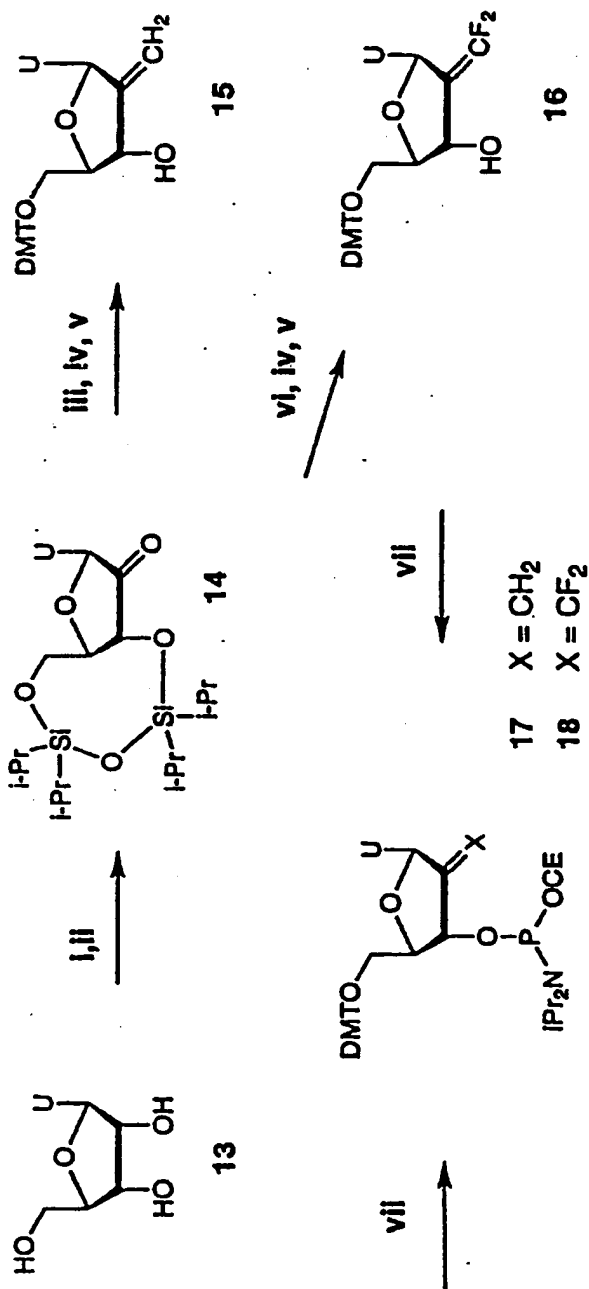


FIG. 82.

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FIG. 83.



i) = Markiewicz reagent
 ii) = DMSO & Ac₂O
 iii) = Ph₃PCH₃I
 iv) = TBAF/THF

v) = DMTCl/Pyr
 vi) = Ph₃P, ClCF₂COONa
 vii) = P(OCE)(N-iPr₂)Cl

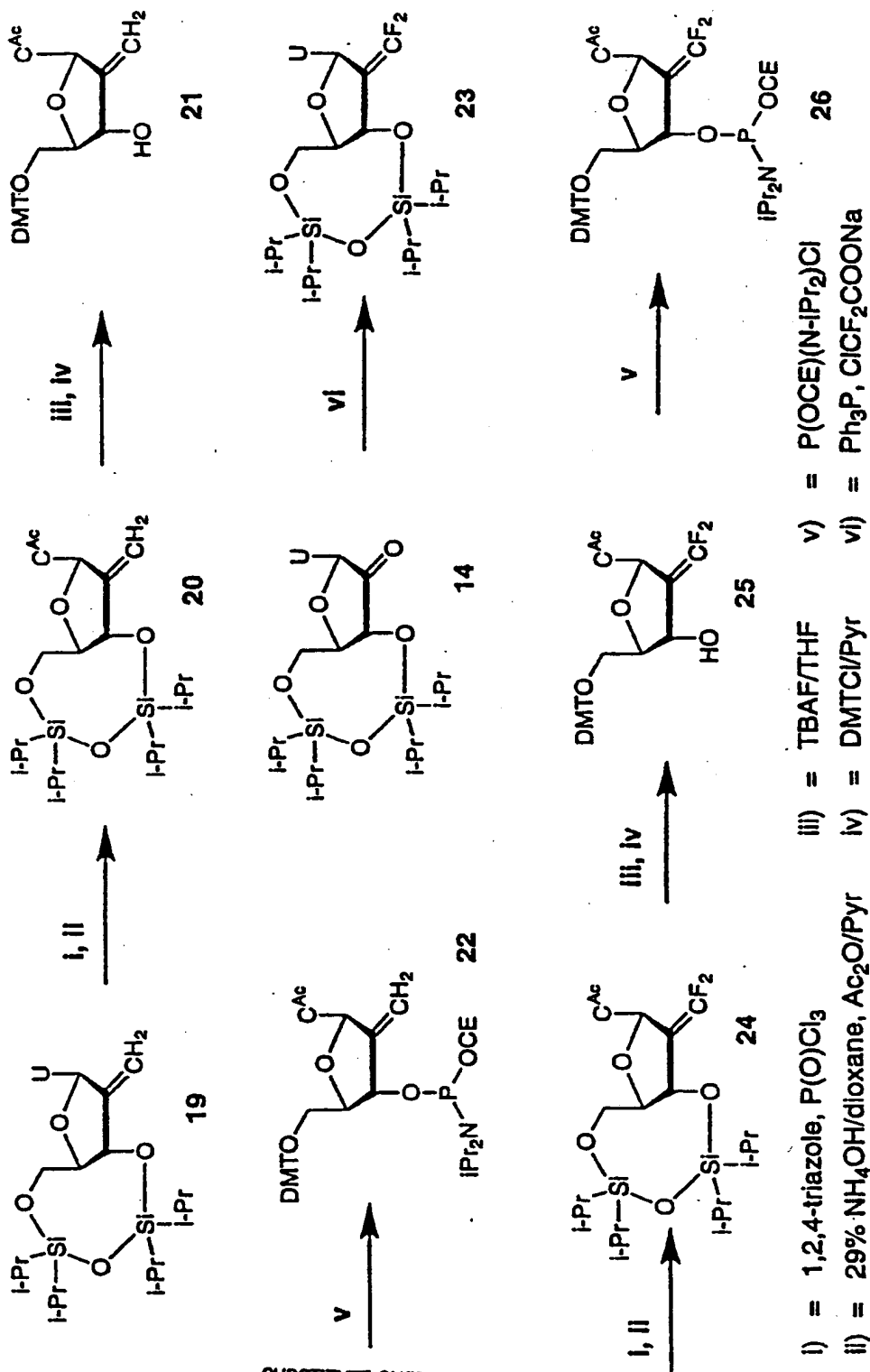
17 X = CH₂
 18 X = CF₂

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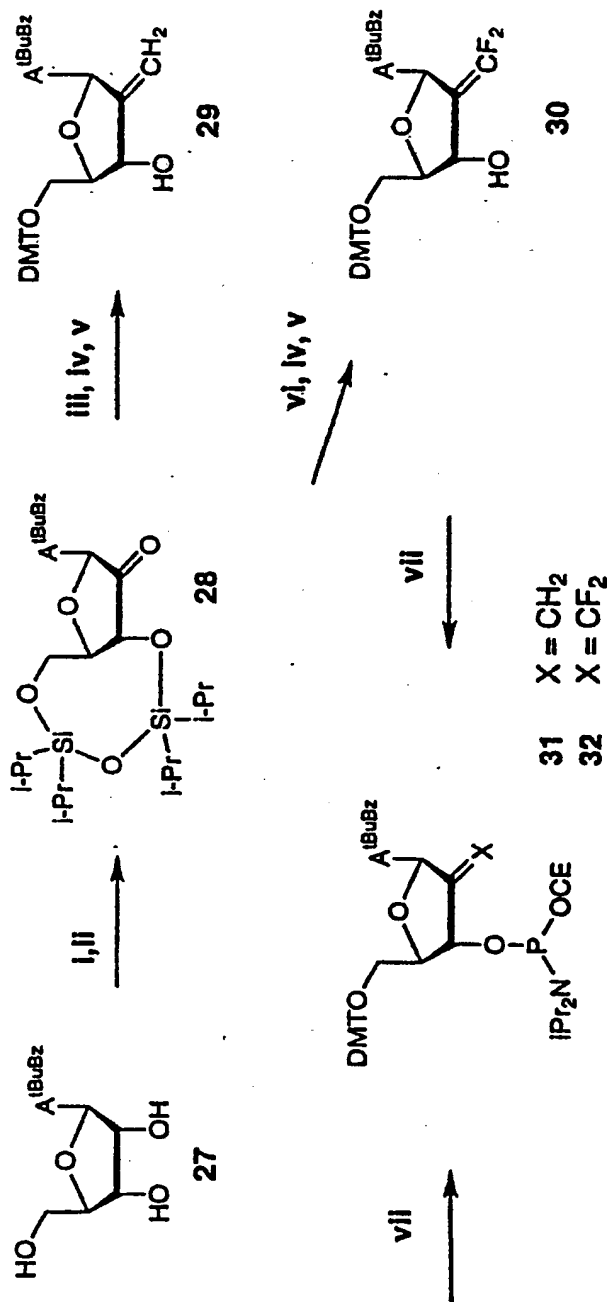
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FIG. 84.



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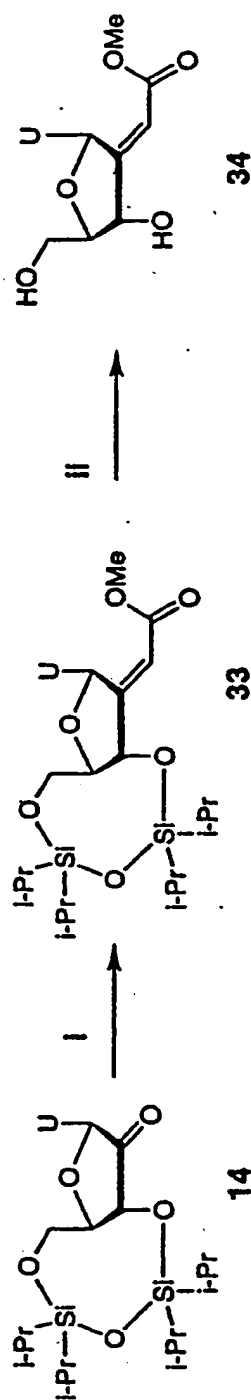
- I) = Markiewicz reagent
 II) = DMSO & Ac_2O
 III) = $\text{Ph}_3\text{PCH}_3\text{I}$
 IV) = TBAF/THF
 V) = DMTCI/Pyr
 VI) = Ph_3P , $\text{ClCF}_2\text{COONa}$
 VII) = $\text{P}(\text{OCE})(\text{N-IPt}_2)\text{Cl}$

FIG. 85.

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- i) = $\text{Ph}_3\text{PC}=\text{CHC}(\text{O})\text{OCH}_3 \cdot \text{OAc}$
- ii) = $\text{NEt}_3 \cdot 3 \text{ HF}$
- iii) = DMTCI/Pyr
- iv) = $\text{P}(\text{OCE})(\text{N-IPr}_2)\text{Cl}$
- v) = MeOH/NaOH

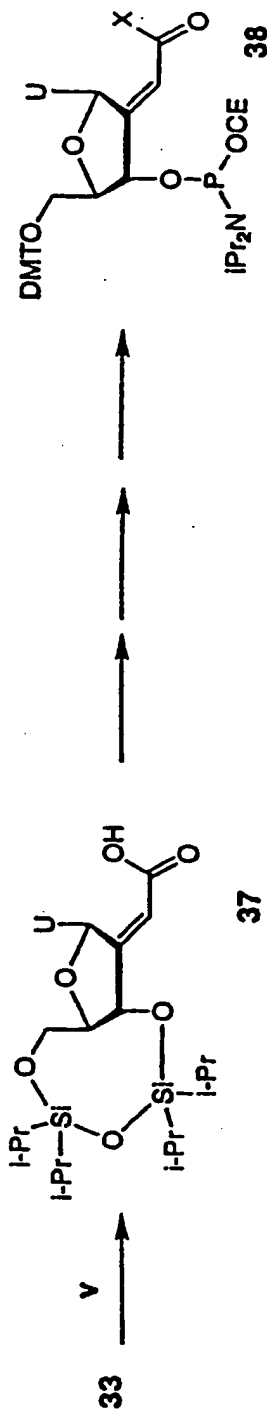
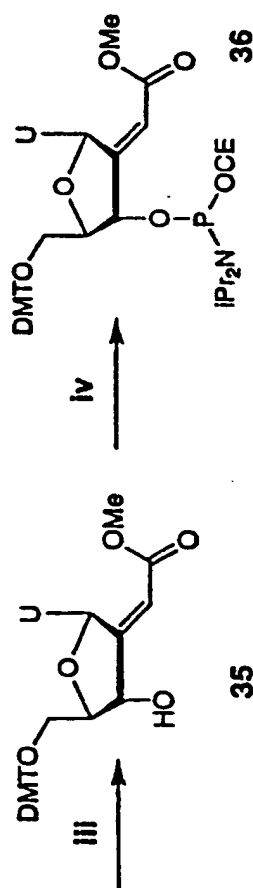
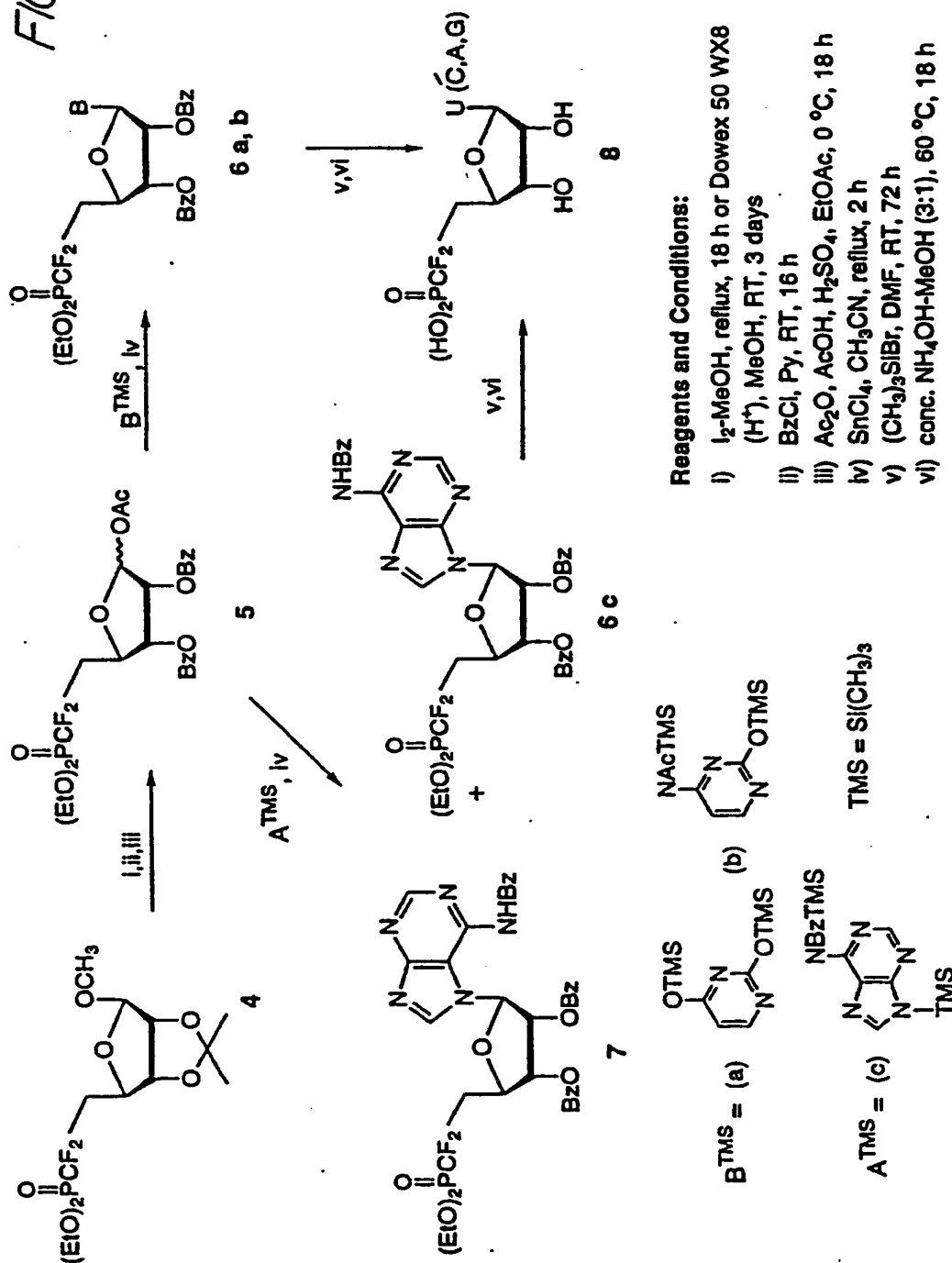


FIG. 86.

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FIG. 87.

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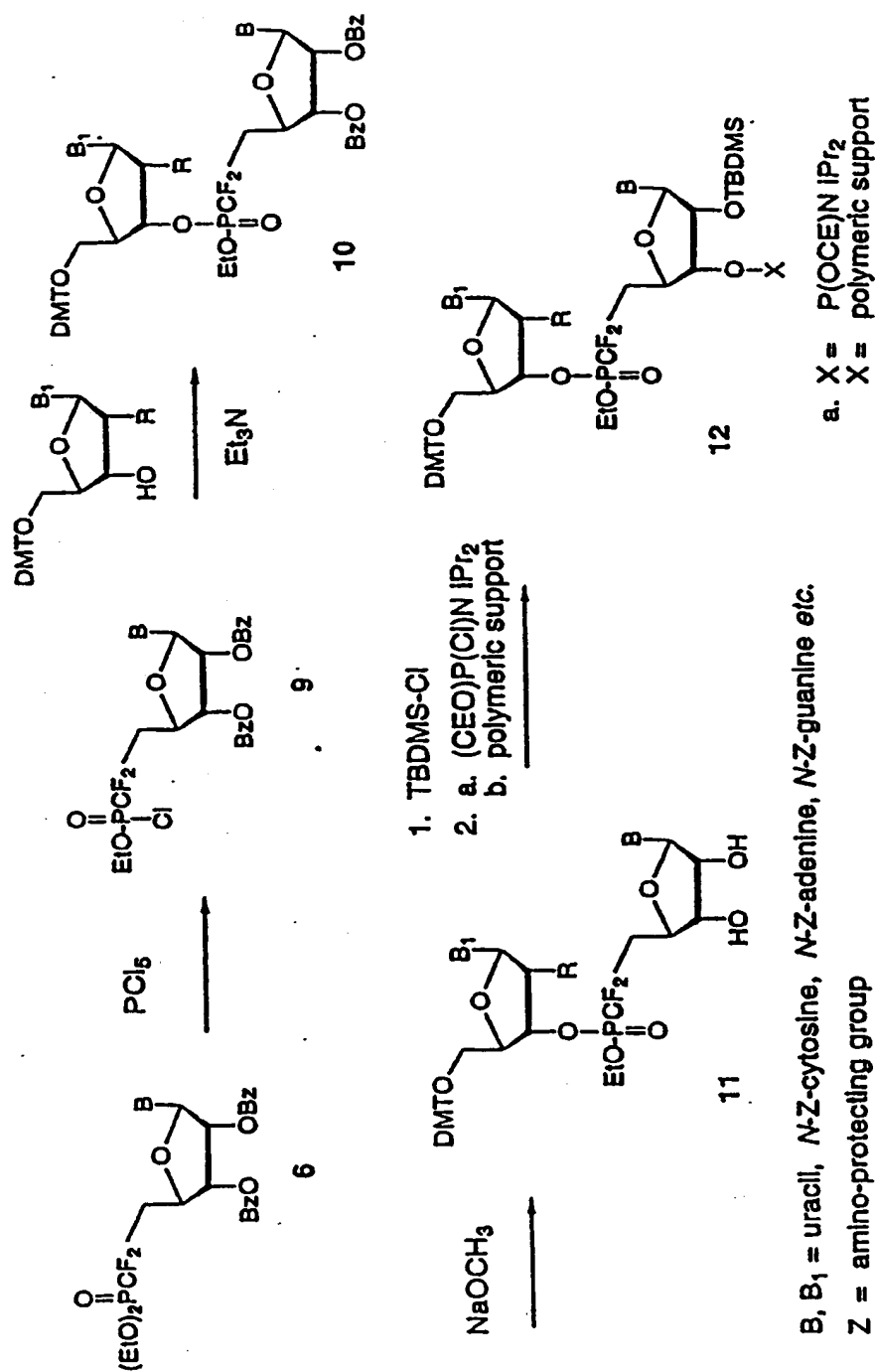
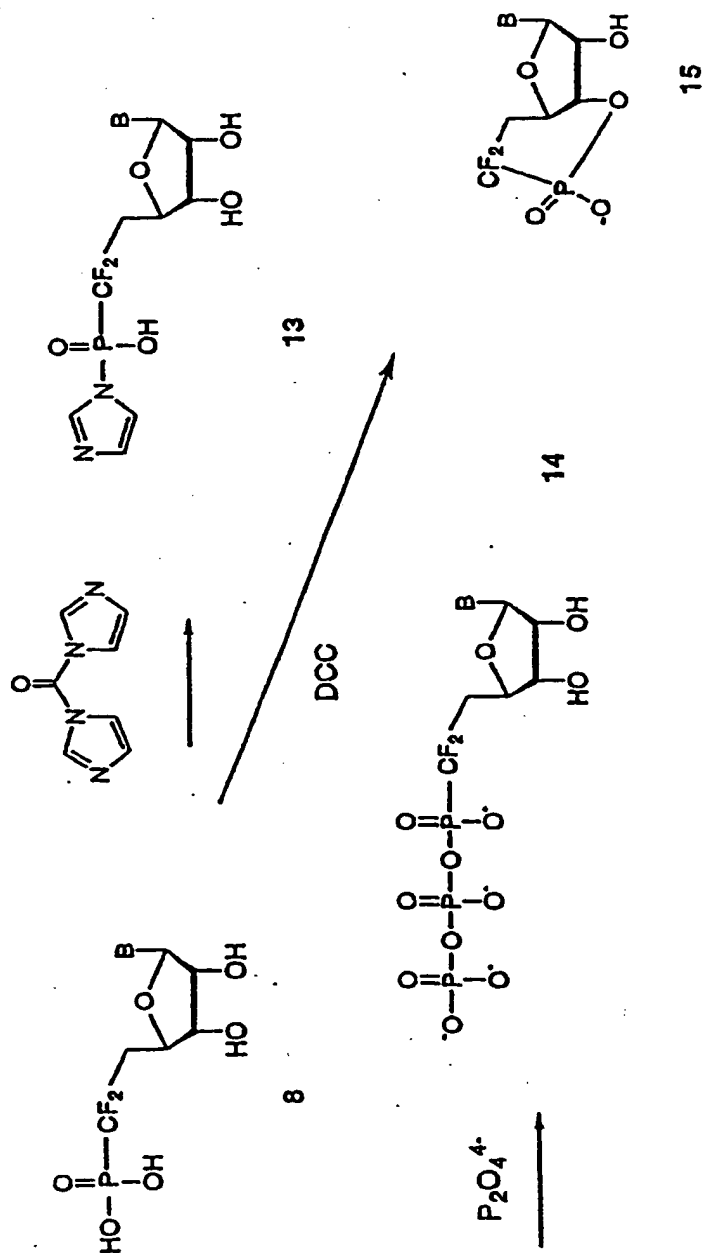


FIG. 88.

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B = uracil, N-Z-cytosine, N-Z-adenine, N-Z-guanine etc.

Z = amino-protecting group

FIG. 89.

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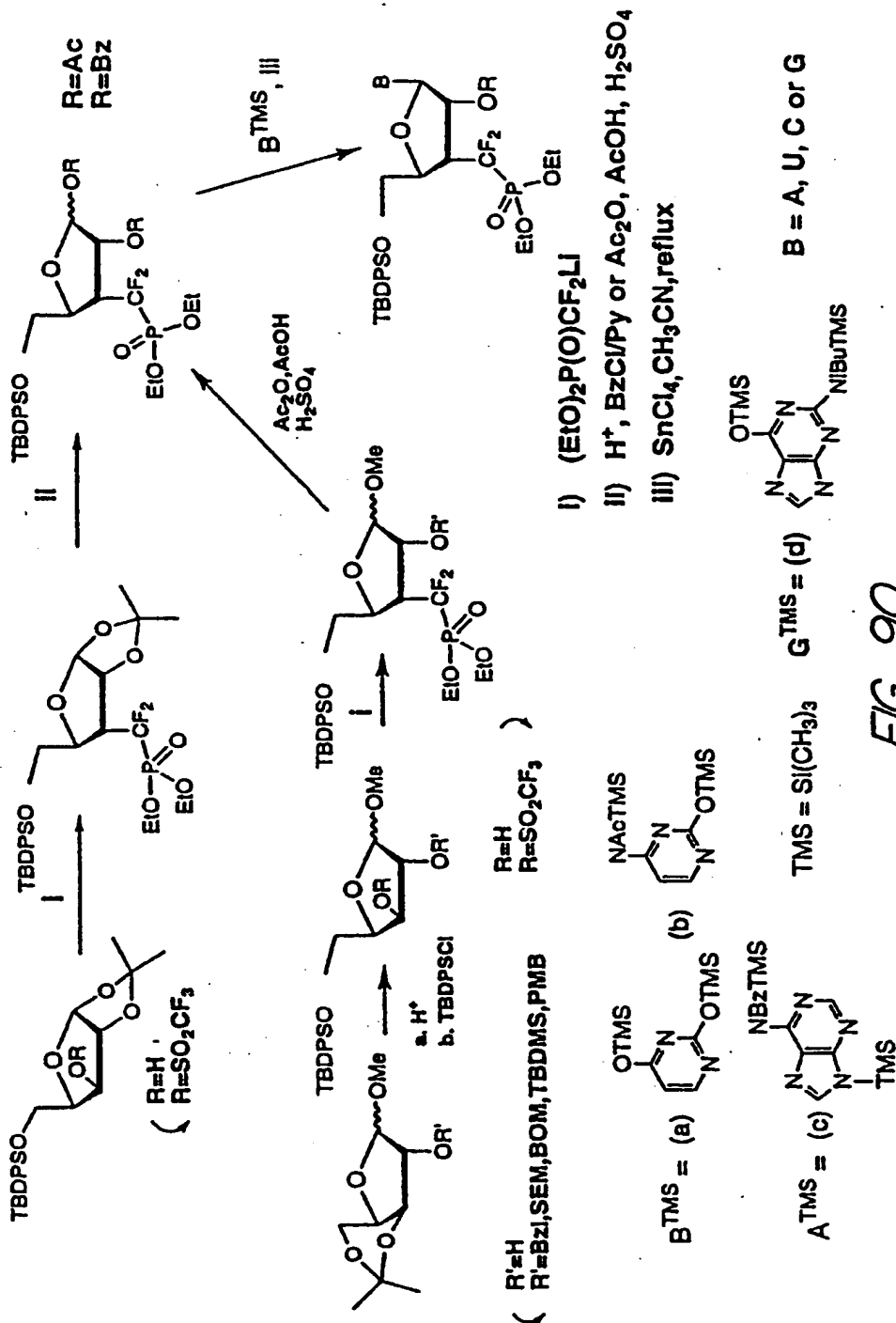


FIG. 90.

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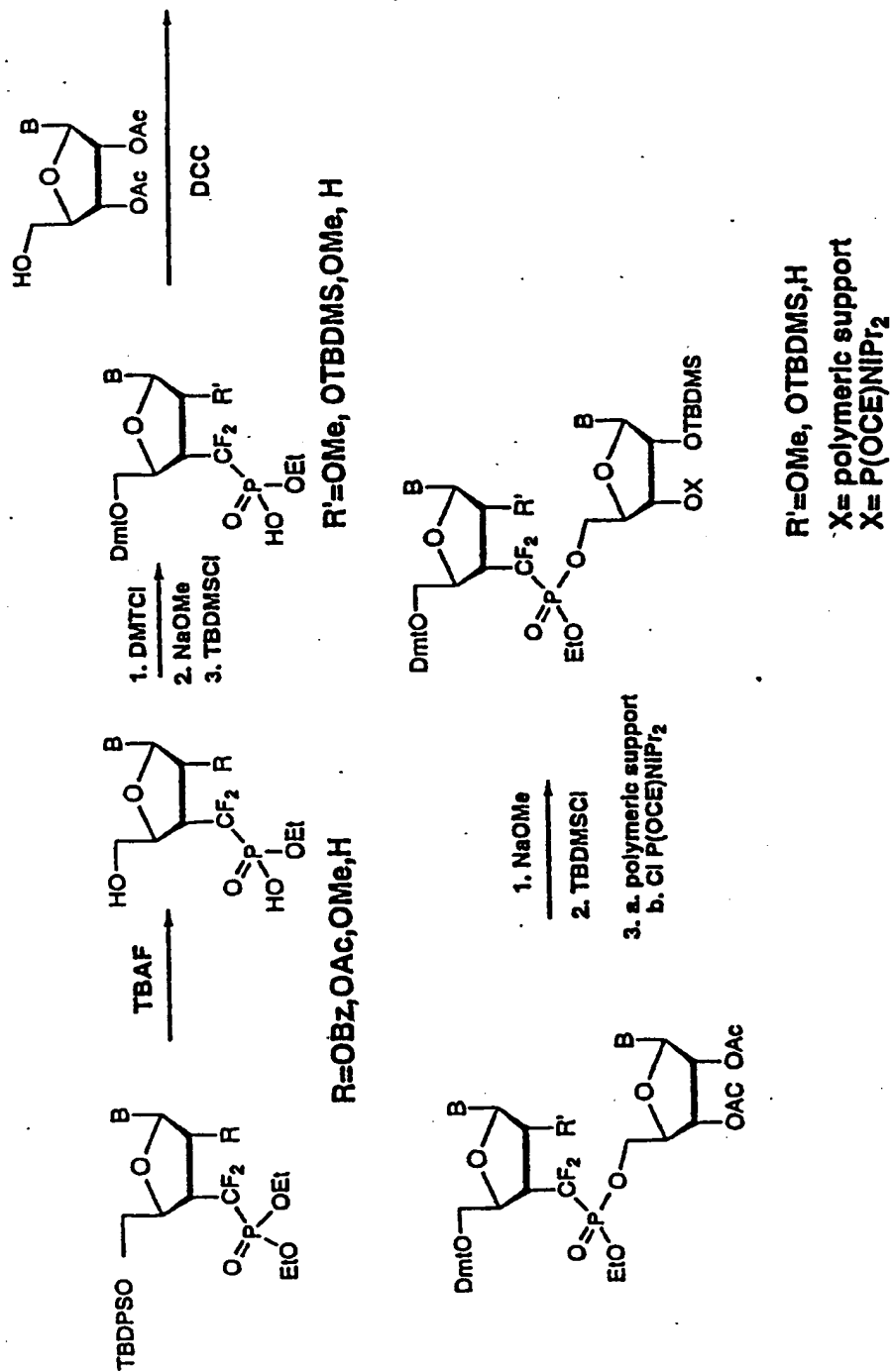
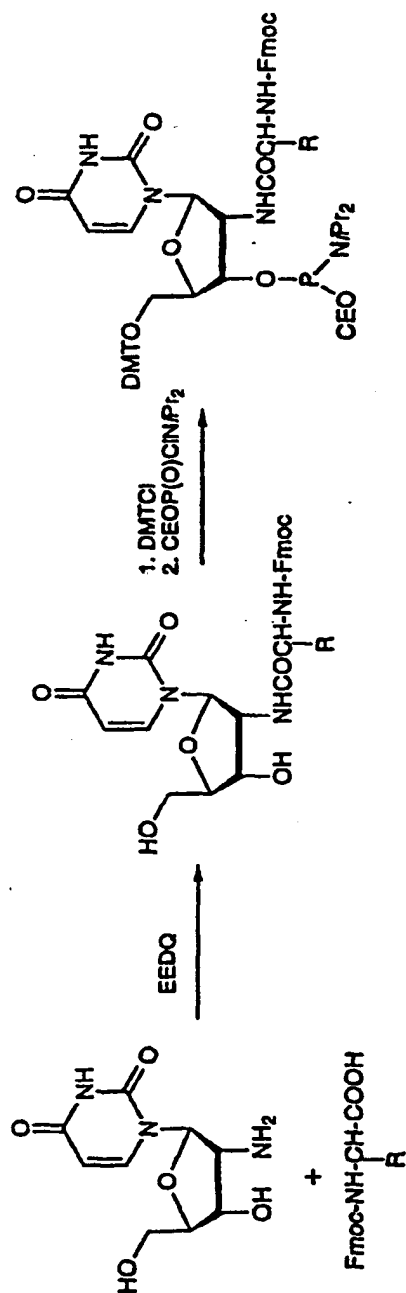


FIG. 91.

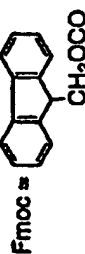
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SUBSTITUTE SHEET (RULE 26)

EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R = CH₃, CH₂-C₆H₄ (phe), (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBzl
 (ala) (lys) (asp)

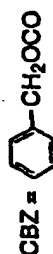
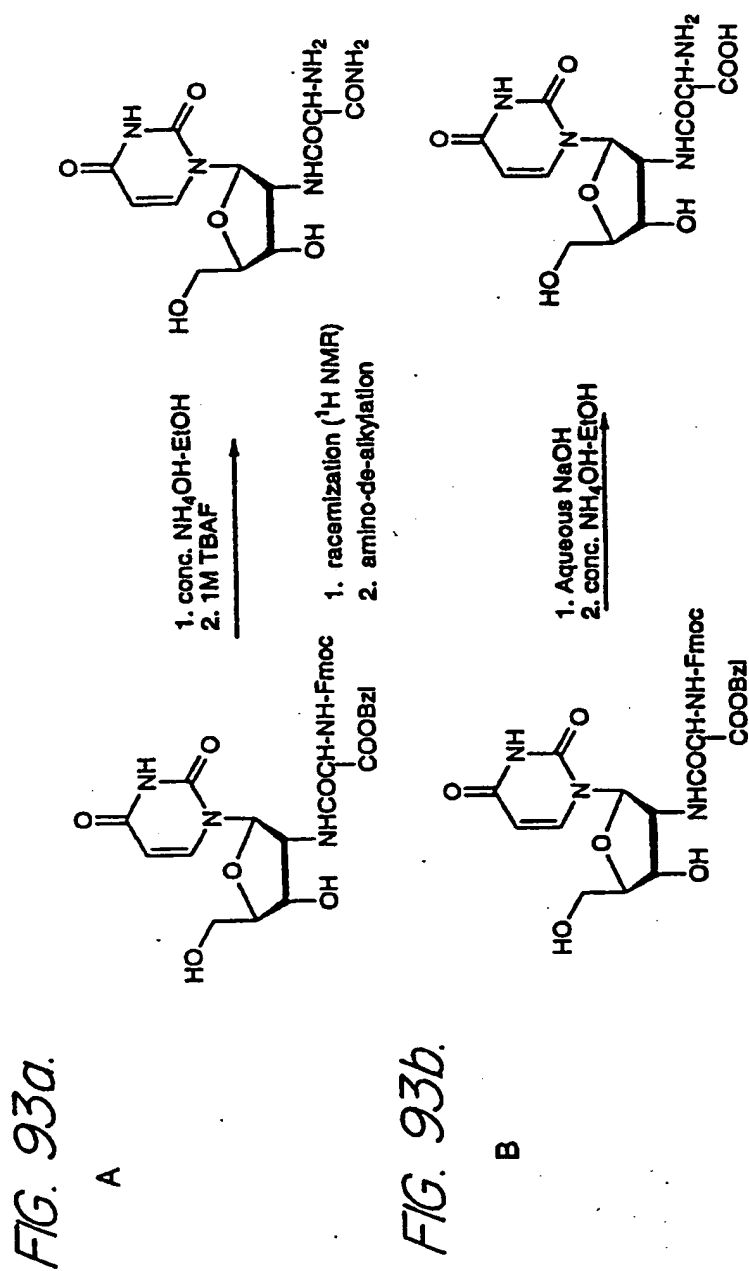


FIG. 92.

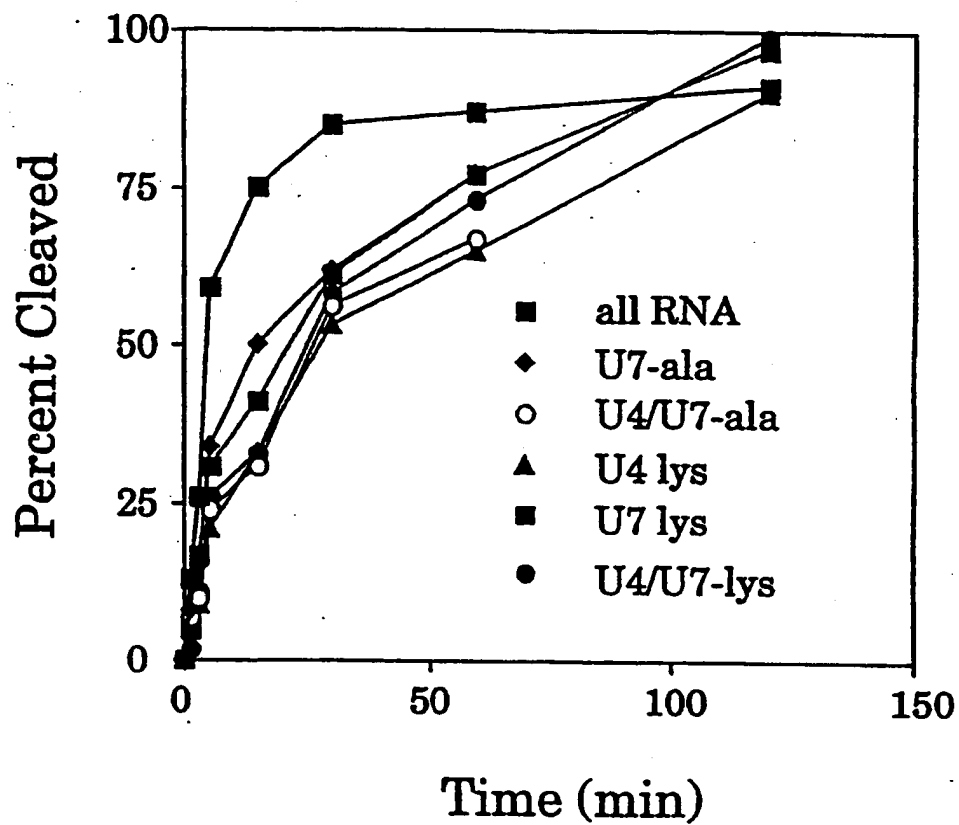
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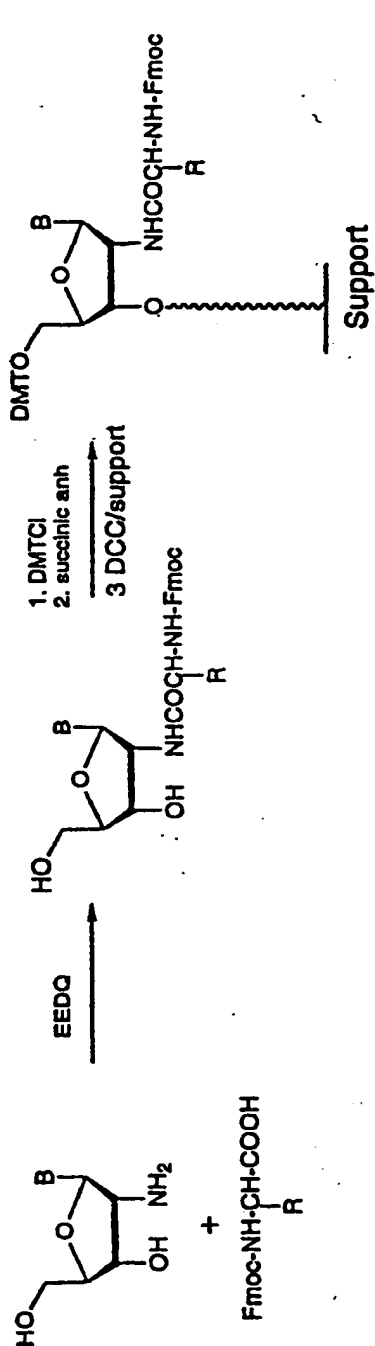
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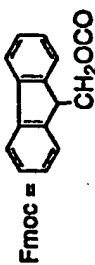
[Ribozyme] = 40 nM [Substrate] = ~1nM

FIG. 95.


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EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R = CH₃, CH₂- (phe), (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBzl (asp)

CBZ = -CH₂OCO

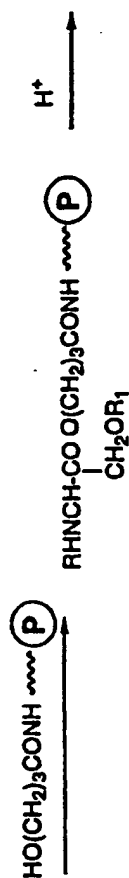
Bzl = -CH₂

B = Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

FIG. 96.

SUBSTITUTE SHEET (RULE 26)

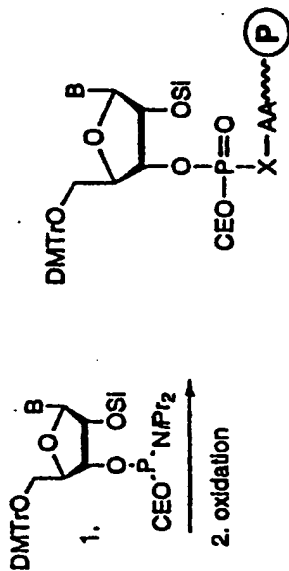
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a R=Fmoc, R₁=DMTr
b R=MMTr, R₁=Bz



a R=Fmoc, R₁=H
b R=H, R₁=Bz



a X=O, AA=CH₂CH(NHFmoc)CO
b X=NH, AA=CH(CH₂OBz)CO

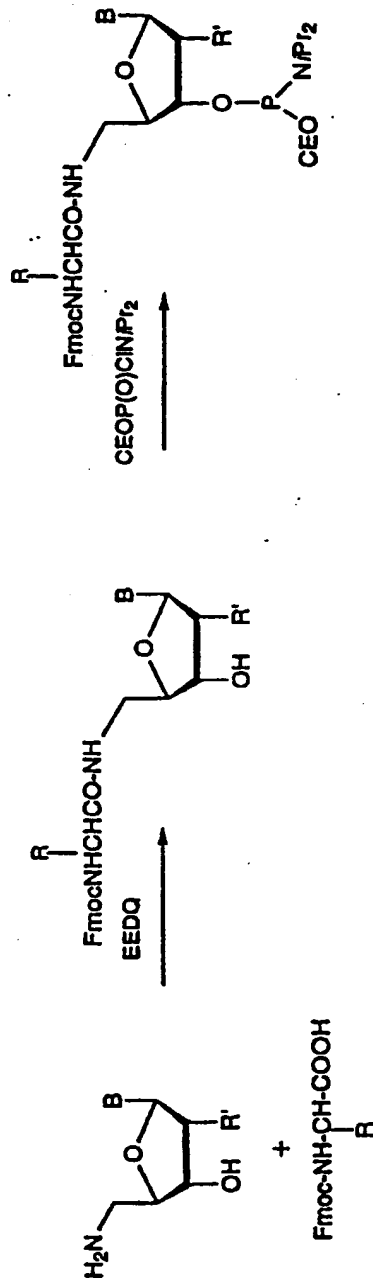
B= Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

FIG. 97.

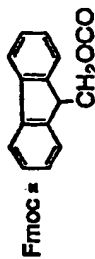
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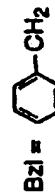
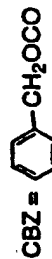
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EEDQ = N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline



R = CH₃, CH₂-C₆H₄-C(=O)OCH₃ (ala), (CH₂)₄NH-Fmoc, (CH₂)₄NH-CBZ, CH₂COOBzl (lys), (asp)



R' = H, OMe, OTBDMSI

B = Ura, Cyt^{bz}, Ade^{bz}, Gua^{ibu}, mod. base, H

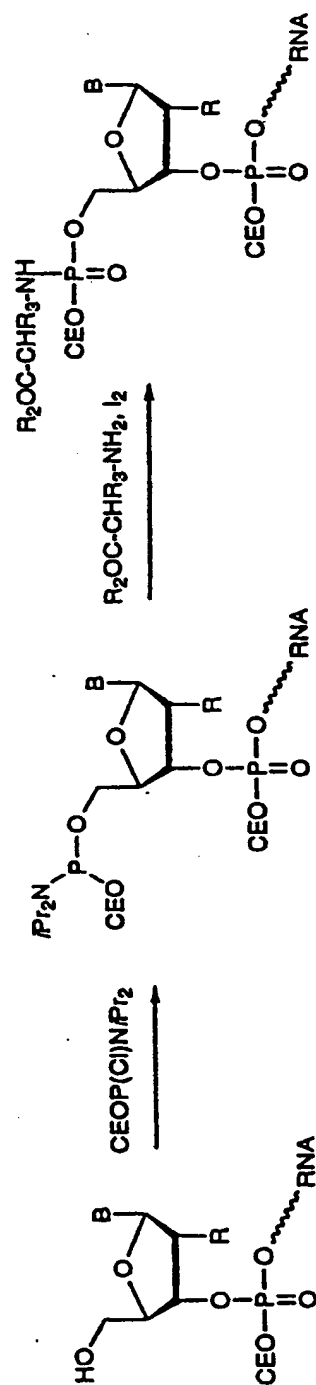
FIG. 98.

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FIG. 99.



$B = \text{Ura, Cyt}^{\text{bz}}, \text{Ade}^{\text{bz}}, \text{Gua}^{\text{ibu}}, \text{mod. base, H}$
 $R = \text{H, OCH}_3, \text{OTBDMS, Hal, NHR}_1$
 $R_2 = \text{OBzl, peptidyl}$

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FIG. 100.

Reversion of mutant RNA

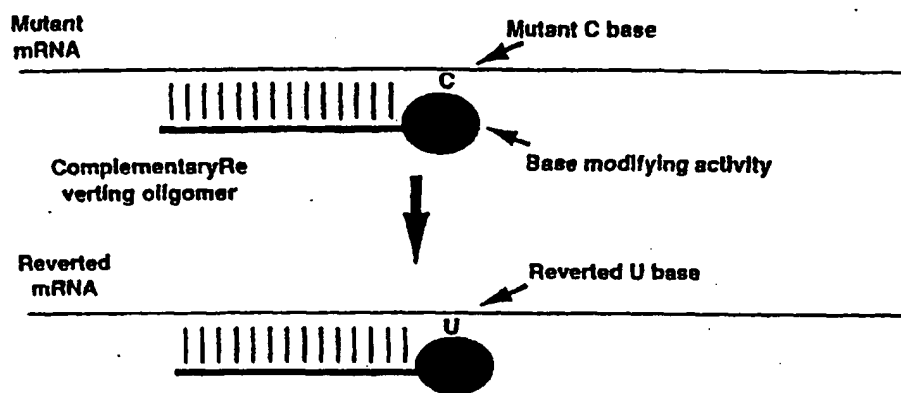
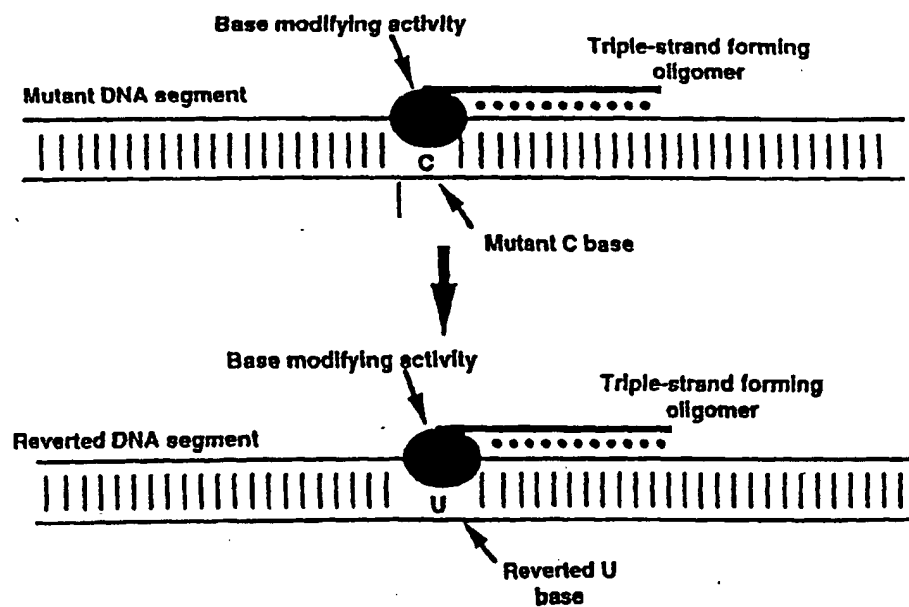


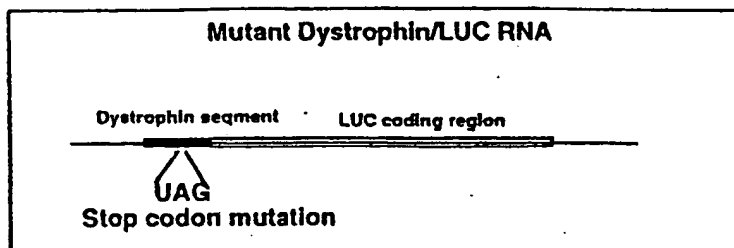
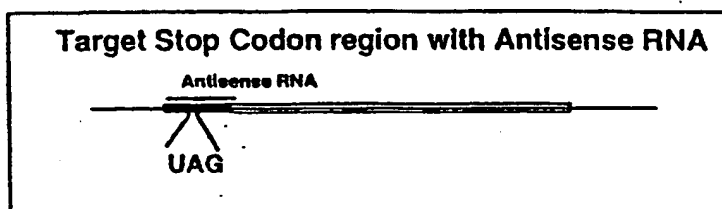
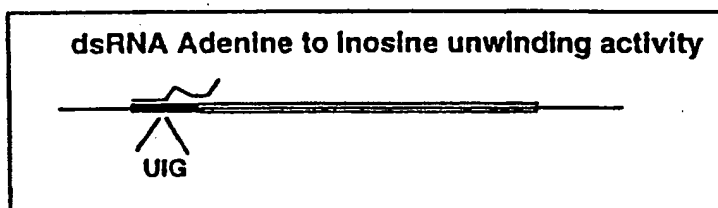
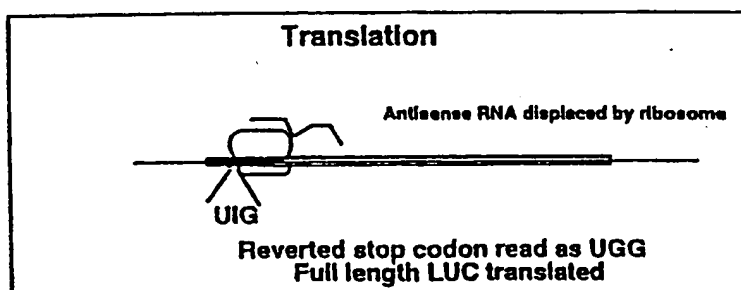
FIG. 101.

Reversion of mutant DNA



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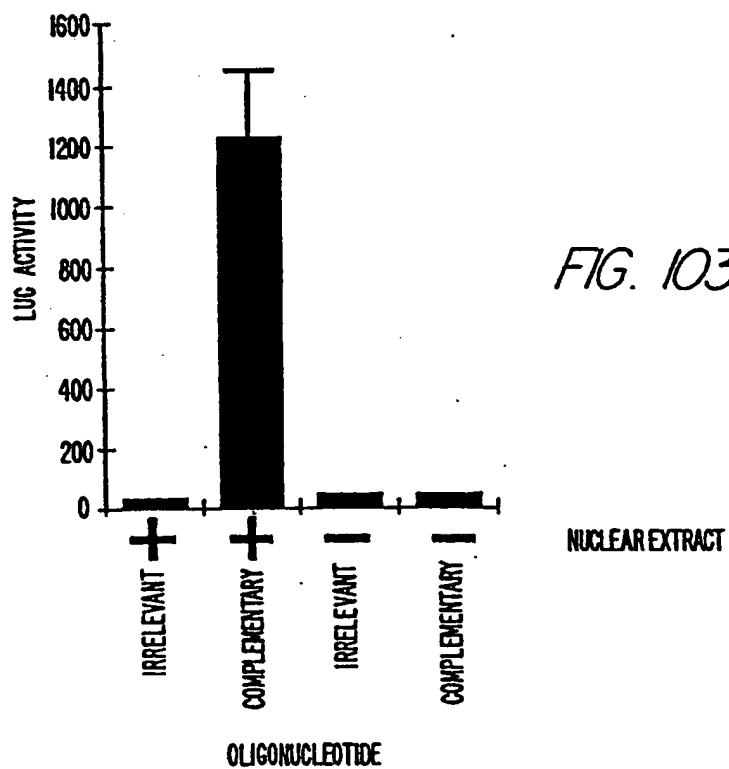
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*FIG. 102a.**FIG. 102b.**FIG. 102c.**FIG. 102d.*

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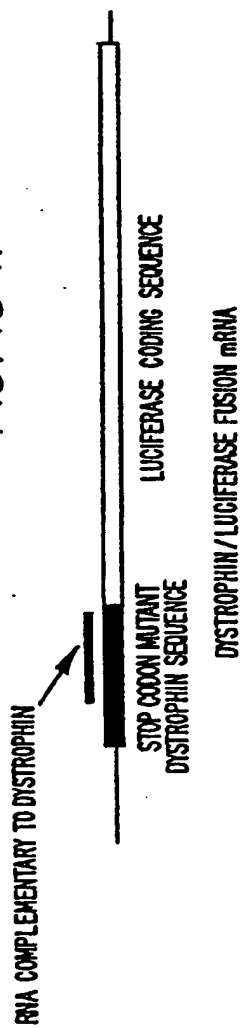


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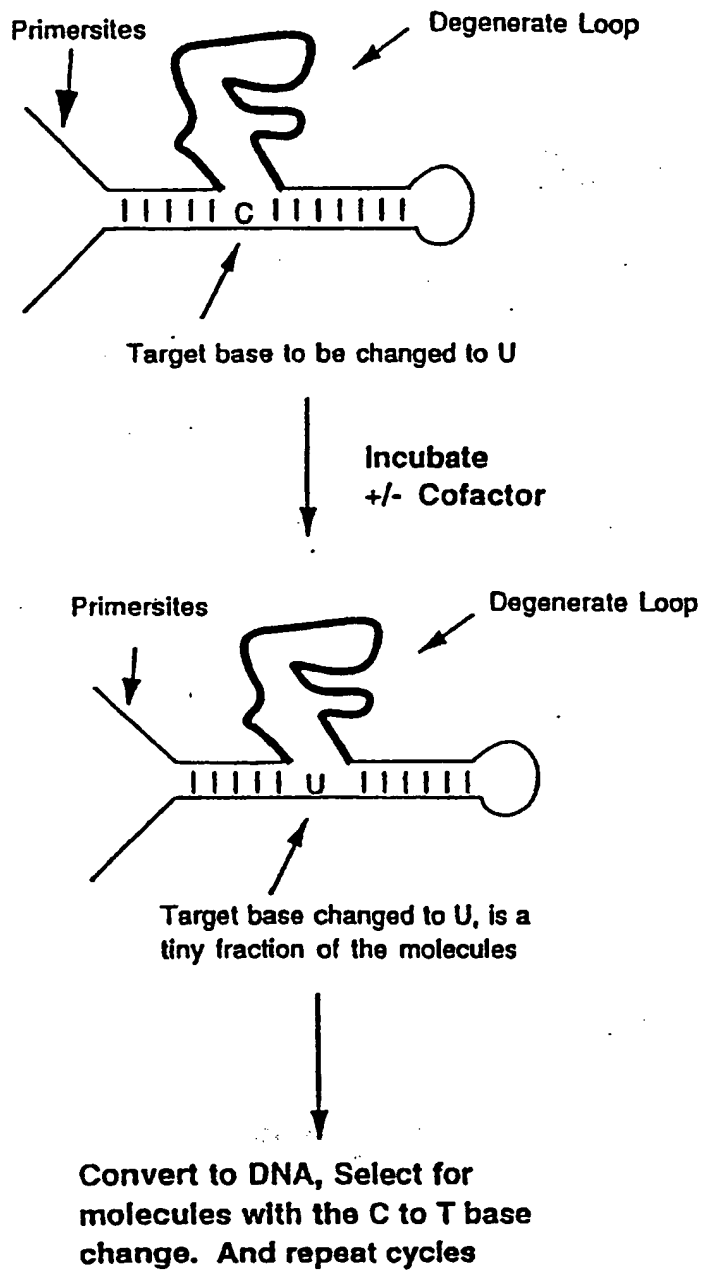
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FIG. 104.



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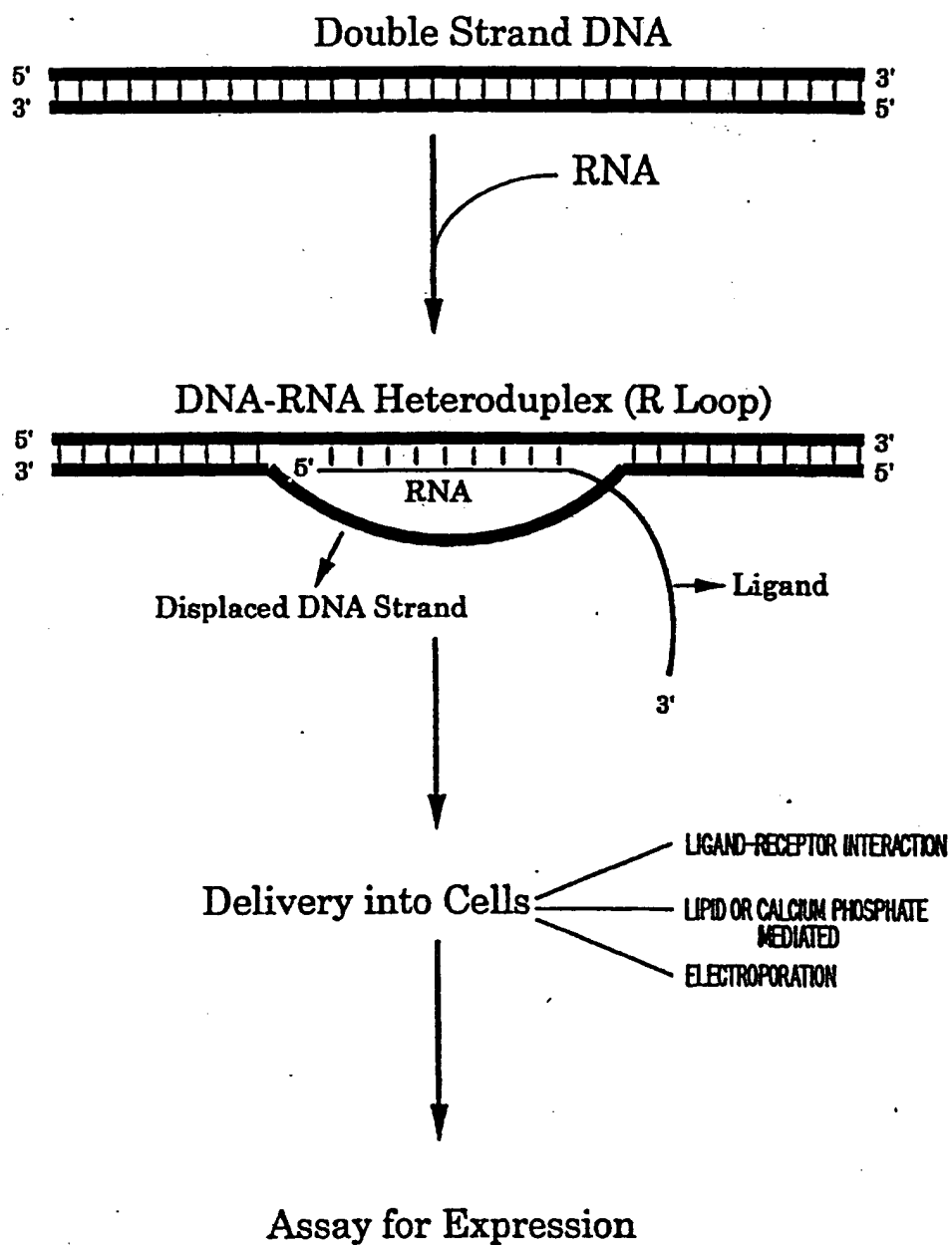
FIG. 105.



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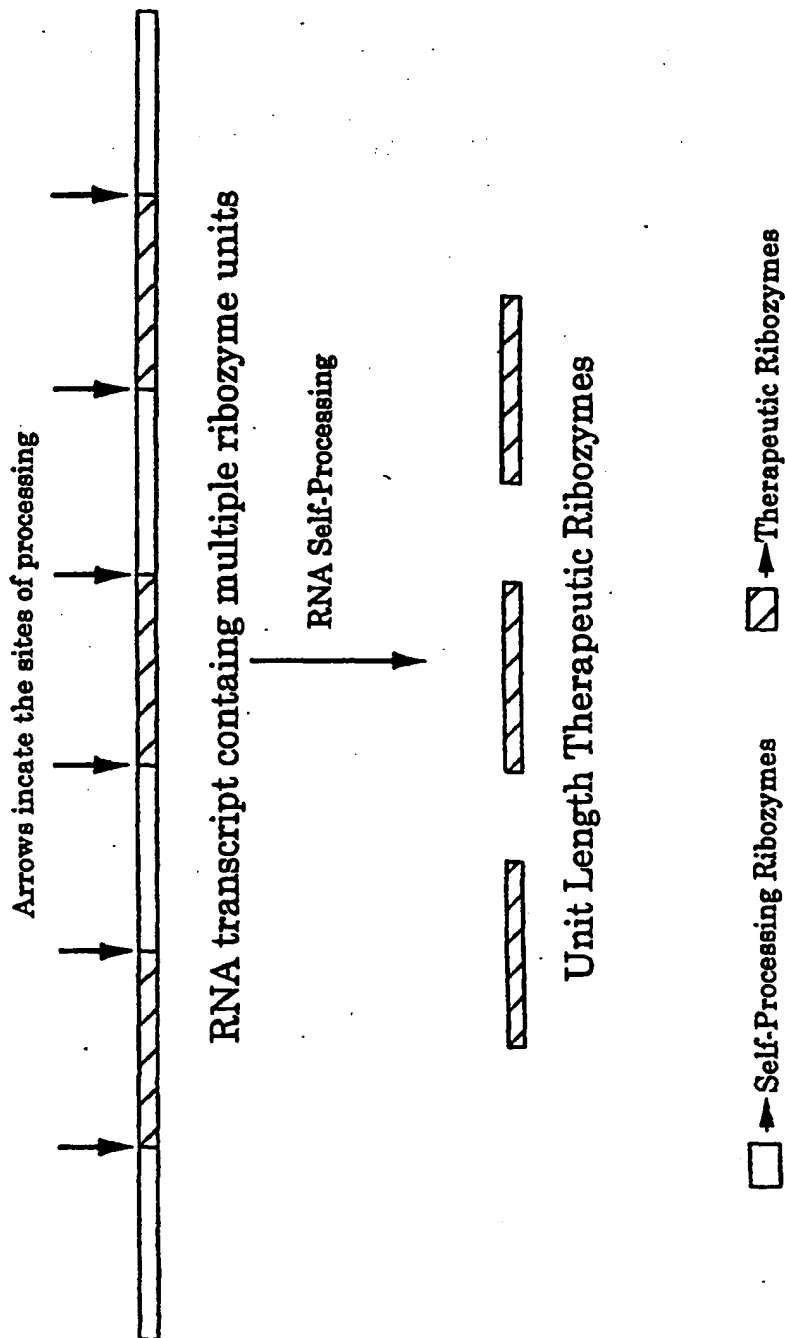
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*FIG. 106.*

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FIG. 107



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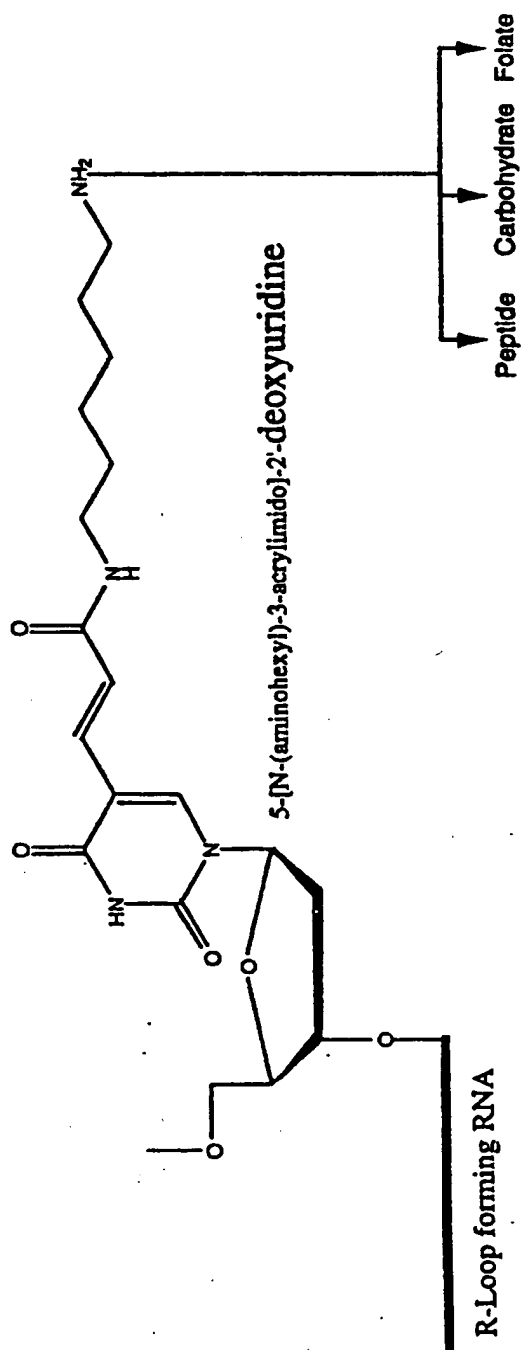


FIG. 108.

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